Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	5327	glucoamylase$1	US-PGPUB; USPAT	OR	OFF	2004/05/13 16:46
L2	314	EMERSONII OR TALAROMYCES	US-PGPUB; USPAT	OR	OFF	2004/05/13 16:46
L3	10901	thermostab$	US-PGPUB; USPAT	OR	OFF	2004/05/13 16:47
L4	24	l1 same l2	US-PGPUB; USPAT	OR	OFF	2004/05/13 16:47
L5	41	l1 near3 l3	US-PGPUB; USPAT	OR	OFF	2004/05/13 16:47
L6	113331	(ACTIVIT$5 NEAR4 (INCREAS$ OR HIGH$5))	US-PGPUB; USPAT	OR	OFF	2004/05/13 16:48
L7	64	l1 near5 l6	US-PGPUB; USPAT	OR	OFF	2004/05/13 16:48
L8	22	l3 and l7	US-PGPUB; USPAT	OR	OFF	2004/05/13 16:48
L9	37	l4 or l8	US-PGPUB; USPAT	OR	OFF	2004/05/13 16:49

PGPUB-DOCUMENT-NUMBER: 20040091983

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040091983 A1

TITLE: Secondary liquefaction in ethanol production

PUBLICATION-DATE: May 13, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Veit, Christopher	Wake Forest	NC	US	
Felby, Claus	Vekso		DK	
Fuglsang, Claus Crone	Niva		DK	

APPL-NO: 10/ 416393

DATE FILED: May 9, 2003

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	PA 2000 01676	2000DK-PA 2000 01676	November 10, 2000
DK	PA 2000 01854	2000DK-PA 2000 01854	December 11, 2000

PCT-DATA:
APPL-NO: PCT/DK01/00737
DATE-FILED: Nov 9, 2001
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/161

ABSTRACT:

The invention relates to a method of producing ethanol by fermentation, said method comprising a secondary liquefaction step in the presence of a thermostable acid alpha-amylase or, a thermostable maltogenic acid alpha-amylase.

---------- KWIC ---------

Detail Description Paragraph - DETX (133):
[0141] Other contemplated Aspergillus glucoamylase variants include variants to enhance the thermal stability: G137A and G139A (Chen et al. (1996), Prot. Engng. 9, 499-505); D257E and D293E/Q (Chen et al. (1995), Prot. Engng. 8, 575-582); N182 (Chen et al. (1994), Biochem. J. 301, 275-281); disulphide bonds, A246C (Fierobe et al. (1996), Biochemistry, 35, 8698-8704; and introduction of Pro residues in position A435 and S436 (Li et al. (1997), Protein Engng. 10, 1199-1204. Furthermore, Clark Ford presented a paper on Oct. 17, 1997, ENZYME ENGINEERING 14, Beijing/China Oct 12-17, 1997, Abstract number: Abstract book p.0-61. The abstract suggests mutations in positions G137A, N20C/A27C, and S30P in an Aspergillus awamori glucoamylase to improve the thermal stability. Other glucoamylases include Talaromyces glucoamylases.

in particular derived from Talaromyces emersonii (WO 99/28448), Talaromyces leycettanus (U.S. Pat. No. Re. 32,153), Talaromyces duponti, Talaromyces thermopiles (U.S. Pat. No. 4,587,215). Bacterial glucoamylases contemplated include glucoamylases from the genus Clostridium, in particular C. thermoamylolyticum (EP 135,138), and C. thermohydrosulfuricum (WO 86/01831).

Claims Text - CLTX (25):
25. The method of claim 23, wherein the glucoamylase is microbial, such as, e.g., derived from a strain of Aspergillus niger or Talaromyces emersonii.

PGPUB-DOCUMENT-NUMBER: 20040063184

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040063184 A1

TITLE: Fermentation processes and compositions

PUBLICATION-DATE: April 1, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Grichko, Varvara	Raleigh	NC	US	

APPL-NO: 10/ 459143

DATE FILED: June 10, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60413730 20020926 US

US-CL-CURRENT: 435/161, 435/105

ABSTRACT:

The present invention provides improved fermentation processes, including for use in an ethanol production process. The improved fermentation processes include applying esterases (such as, lipases, phospholipases and cutinases), laccases, phytases and/or proteases to a fermentation process. The improved fermentation process may also involve the addition of various growth stimulators for the fermenting microorganisms, including vitamins and mineral.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. provisional application No. 60/413,730 filed Sep. 26, 2002, the contents of which are fully incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (69):

[0066] Other Aspergillus glucoamylase variants include variants to enhance the thermal stability, such as, G137A and G139A (Chen et al. (1996), Prot. Engng. 9,499-505); D257E and D293E/Q (Chen et al. (1995), Prot. Engng. 8, 575-582); N182 (Chen et al. (1994), Biochem. J. 301, 275281); disulphide bonds, A246C (Fierobe et al. (1996), Biochemistry, 35, 8698-8704; and introduction of Pro residues in position A435 and S436 (Li et al. (1997), Protein Engng. 10, 1199-1204. Other glucoamylases include Talaromyces glucoamylases, in particular, derived from Talaromyces emersonii (WO 99/28448), Talaromyces leycettanus (U.S. Pat. No. Re. 32,153), Talaromyces duponti, Talaromyces thermophilus (U.S. Pat. No. 4,587,215). Bacterial glucoamylases contemplated include glucoamylases from the genus Clostridium, in particular C. thermoamylolyticum (EP 135,138), and C. thermohydrosulfuricum (WO 86/01831).

PGPUB-DOCUMENT-NUMBER: 20040023349

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040023349 A1

TITLE: Processes for making ethanol

PUBLICATION-DATE: February 5, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bisgaard-Frantzen, Henrik	Bagsvaerd	NC	DK	
Henderson, Lori	Wake Forest		US	

APPL-NO: 10/ 460455

DATE FILED: June 12, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60388488 20020613 US

US-CL-CURRENT: 435/161

ABSTRACT:

The present invention provides improved processes for recovering components of distillers' grain, such as, the components of distillers' dried grain (DDG), for use in various applications, including in the production of ethanol.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. provisional application No. 60/388,488 filed Jun. 13, 2002, the contents of which are fully incorporated herein by reference

---------- KWIC ---------

Detail Description Paragraph - DETX (25):

[0038] Other Aspergillus glucoamylase variants include variants to enhance the thermal stability: G137A and G139A (Chen et al. (1996), Prot. Engng. 9, 499-505); D257E and D293E/Q (Chen et al. (1995), Prot. Engng. 8, 575-582); N182 (Chen et al. (1994), Biochem. J. 301, 275-281); disulphide bonds, A246C (Fierobe et al. (1996), Biochemistry, 35, 8698-8704; and introduction of Pro residues in position A435 and S436 (Li et al. (1997), Protein Engng. 10, 1199-1024. Other glucoamylases include Talaromyces glucoamylases, in particular, derived from Talaromyces emersonii (WO 99/28448), Talaromyces leycettanus (U.S. Pat. No. Re. 32,153), Talaromyces duponti, Talaromyces thermophilus (U.S. Pat. No. 4,587,215). Bacterial glucoamylases contemplated include glucoamylases from the genus Clostridium, in particular C. thermoamylolyticum (EP 135,138), and C. thermohydrosulfuricum (WO 86/01831).

PGPUB-DOCUMENT-NUMBER: 20040002142

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040002142 A1

TITLE: Glucoamylase variants

PUBLICATION-DATE: January 1, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Nielsen, Bjarne Ronfeldt	Virum		DK	
Svendsen, Allan	Horsholm		DK	
Pedersen, Henrik	Bagsvared		DK	
Vind, Jesper	Lyngby		DK	
Hendriksen, Hanne Vang	Holte		DK	
Frandsen, Torben Peter	Frederiksberg C		DK	

APPL-NO: 10/ 421586

DATE FILED: April 23, 2003

RELATED-US-APPL-DATA:

child 10421586 A1 20030423

parent continuation-of 09612489 20000707 US ABANDONED

non-provisional-of-provisional 60143313 19990712 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	1999 00999	1999DK-1999 00999	July 9, 1999

US-CL-CURRENT: 435/101, 435/105 , 435/115 , 435/126 , 435/144 , 435/161 , 435/205 , 435/254.3 , 435/320.1 , 536/23.2

ABSTRACT:

The invention relates to a variant of a parent fungal glucoamylase, which exhibits altered properties, in particular improved thermal stability and/or increased specific activity.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. application Ser. No. 09/612,489 filed Jul. 7, 2000, and claims the benefit under 35 U.S.C. 119 of of U.S. provisional application No. 60/143,313 filed Jul. 12, 1999, and priority of Danish application no. PA 1999 00999, filed Jul. 9, 1999, the contents of which are fully incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (12):
[0010] A term "a thermostable glucoamylase variant" means in the context of the present invention a glucoamylase variant, which has a higher T.sub.1/2

(half-time) in comparison to a corresponding parent glucoamylase. The determination of T1/2 (Method I and Method II) is described below in the "Materials & Methods" section.

Summary of Invention Paragraph - BSTX (13):
[0011] The term "a glucoamylase variant with increased specific activity" means in the context of the present invention a glucoamylase variant with increased specific activity towards the alpha-1,4 linkages in the saccharide in question. The specific activity is determined as k.sub.cat or AGU/mg (measured as described below in the "Materials & Methods" section). An increased specific activity means that the k.sub.cat or AGU/mg values are higher when compared to the k.sub.cat or AGU/mg values, respectively, of the corresponding parent glucoamylase.

Detail Description Paragraph - DETX (2):
[0034] A goal of the work underlying the present invention was to improve the thermal stability and/or increase the specific activity of particular glucoamylases, which are obtainable from fungal organisms, in particular strain of the Aspergillus genus and which themselves had been selected on the basis of their suitable properties in, e.g., starch conversion or alcohol fermentation.

Detail Description Paragraph - DETX (3):
[0035] In this connection, the present inventors have surprisingly found that it is in fact possible to improve the thermal stability and/or increased specific activity of parent glucoamylases by modification of one or more amino acid residues of the amino acid sequence of the parent glucoamylase. The present invention is based on this finding.

Detail Description Paragraph - DETX (6):
[0038] Parent glucoamylase contemplated according to the present invention include wild-type glucoamylases, fungal glucoamylases, in particular fungal glucoamylases obtainable from an Aspergillus strain, such as an Aspergillus niger or Aspergillus awamori glucoamylases and variants or mutants thereof, homologous glucoamylases, and further glucoamylases being structurally and/or functionally similar to SEQ ID NO:2. Specifically contemplated are the Aspergillus niger glucoamylases G1 and G2 disclosed in Boel et al. (1984), "Glucoamylases G1 and G2 from Aspergillus niger are synthesized from two different but closely related mRNAs", EMBO J. 3 (5), p. 1097-1102. The G2 glucoamylase is disclosed in SEQ ID NO: 2. In another embodiment the AMG backbone is derived from Talaromyces, in particular T. emersonii disclosed in WO 99/28448 (See SEQ ID NO: 7 of WO 99/28448).

Detail Description Paragraph - DETX (42):
[0074] All of the variant listed in the section "Glucoamylase variants of the invention" are contemplated to have increased specific activity. Example 3 shows this for a selected variant of the invention.

Detail Description Paragraph - DETX (99):
[0131] By using a thermostable glucoamylase variant of the invention saccharification processes may be carried out at a higher temperature than traditional batch saccharification processes. According to the invention saccharification may be carried out at temperatures in the range from above 60-80.degree. C., preferably 63-75.degree. C. This applied both for traditional batch processes (described above) and for continuous saccharification processes.

Detail Description Paragraph - DETX (100):
[0132] Actually, continuous saccharification processes including one or more membrane separation steps, i.e., filtration steps, must be carried out at

temperatures of above 60.degree. C. to be able to maintain a reasonably high flux over the membrane. Therefore, the thermostable variants of the invention provides the possibility of carrying out large scale continuous saccharification processes at a fair price within and period of time acceptable for industrial saccharification processes. According to the invention the saccharification time may even be shortened.

Detail Description Paragraph - DETX (167):
[0199] Screening for Thermostable AMG Variants

Detail Description Paragraph - DETX (168):
[0200] The libraries are screened in the thermostable filter assay described below.

Detail Description Paragraph - DETX (169):
[0201] Filter Assay for Thermostability

Detail Description Paragraph - DETX (196):
[0224] Construction, by Localized Random, Doped Mutagenesis, of A. niger AMG Variants Having Improved Thermostability Compared to the Parent Enzyme

Detail Description Paragraph - DETX (197):
[0225] To improve the thermostability of the A. niger AMG random mutagenesis in pre-selected region was performed.

Detail Description Paragraph - DETX (243):
[0271] The library was screened in the thermostability filter assays using a Protran filter and incubating at 67-69.degree. C. as described in the "Material & Methods" section above

Detail Description Paragraph - DETX (245):
[0272] Thermostability at 68.degree. C.

Detail Description Paragraph - DETX (247):
[0274] The thermostability was determined as T1/2 using Method I at 68.degree. C. as described in the "Materials & Methods" section and compared to the wild-type A. niger AMG G2 under the same conditions.

Detail Description Paragraph - DETX (252):
[0277] Thermostability at 75.degree. C.

Detail Description Paragraph - DETX (254):
[0279] The thermostability was determined as T1/2 using method I at 75.degree. C., ph 4.5, as described in the "Materials & Methods" section and compared to the wild-type A. niger AMG G2 under the same conditions.

Detail Description Paragraph - DETX (257):
[0281] Saccharification performance of the variant AGR 130 (V59A+L66R+T72I+S119P+N313G+S340G+S356G+A393R+Y402F+E408R+N427M) having improved thermostability (see Example 4) is tested at 70.degree. C. described below.

Claims Text - CLTX (5):
5. The variant of any of claims 1-4, wherein the parent glucoamylase is obtained from the genus Aspergillus, in particular A. niger, or Talaromyces, in particular Talaromyces emersonii.

Claims Text - CLTX (11):
11. The variant of any of claims 1-10, wherein the variant has increased

specific activity when compared with the parent glucoamylase.

PGPUB-DOCUMENT-NUMBER: 20040002136

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040002136 A1

TITLE: Transformation system in the field of filamentous fungal hosts

PUBLICATION-DATE: January 1, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Emalfarb, Mark Aaron	Jupiter	FL	US	
Burlingame, Richard Paul	Manitowoc	WI	US	
Olson, Philip Terry	Manitowoc	WI	US	
Sinitsyn, Arkady	Moscow	WI	RU	
Panteleimonovich	Toulouse		FR	
Parriche, Martine	Quint-Fonsegrives		FR	
Bousson, Jean Christophe	Appleton		US	
Pynnonen, Christine Marie	Houten		NL	
Punt, Peter Jan	Vleuten-De Meern		NL	
Van Zeijl, Cornelia Maria Johanna				

APPL-NO: 10/ 394568

DATE FILED: March 21, 2003

RELATED-US-APPL-DATA:

child 10394568 A1 20030321

parent continuation-of 09548938 20000413 US GRANTED

parent-patent 6573086 US

child 10394568 A1 20030321

parent continuation-in-part-of PCT/EP98/06496 19981006 US UNKNOWN

child 10394568 A1 20030321

parent continuation-in-part-of PCT/NL99/00618 19991006 US UNKNOWN

US-CL-CURRENT: 435/69.1, 435/189 , 435/193 , 435/196 , 435/200 , 435/219 , 435/254.2 , 435/320.1 , 536/23.2

ABSTRACT:

A novel transformation system in the field of filamentous fungal hosts for expressing and secreting heterologous proteins or polypeptides is described. The invention also covers a process for producing large amounts of polypeptide or protein in an economical manner. The system comprises a transformed or transfected fungal strain of the genus Chrysosporium, more particularly of Chrysosporium lucknowense and mutants or derivatives thereof. It also covers transformants containing Chrysosporium coding sequences, as well expression-regulating sequences of Chrysosporium genes. Also provided are

novel fungal enzymes and their encoding sequences and expression-regulating sequences.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (55):
[0051] An expression-regulatingregion is a DNA sequence recognised by the host Chrysosporium strain for expression. It comprises a promoter sequence operably linked to a nucleic acid sequence encoding the polypeptide to be expressed. The promoter is linked such that the positioning vis--vis the initiation codon of the sequence to be expressed allows expression. The promoter sequence can be constitutive or inducible. Any expression regulating sequence or combination thereof capable of permitting expression of a polypeptide from a Chrysosporium strain is envisaged. The expression regulating sequence is suitably a fungal expression-regulating region e.g. an ascomycete regulating region. Suitably the fungal expression regulating region is a regulating region from any of the following genera of fungi: Aspergillus, Trichoderma, Chrysosporium (preferred), Hansenula, Mucor, Pichia, Neurospora, Tolypocladium, Rhizomucor, Fusarium, Penicillium, Saccharomyces, Talaromyces or alternative sexual forms thereof like Emericella, Hypocrea e.g. the cellobiohydrolase promoter from Trichoderma, glucoamylase promoter from Aspergillus, glyceraldehyde phosphate dehydrogenase promoter from Aspergillus, alcohol dehydrogenase A and alcohol dehydrogenase R promoter of Aspergillus, TAKA amylase promoter from Aspergillus, phosphoglycerate and cross-pathway control promoters of Neurospora, aspartic proteinase promoter of Rhizomucor miehei, lipase promoter of Rhizomucor miehei and beta-galactosidase promoter of Penicillium canescens. An expression regulating sequence from the same genus as the host strain is extremely suitable, as it is most likely to be specifically adapted to the specific host. Thus preferably the expression regulating sequence is one from a Chrysosporium strain.

Summary of Invention Paragraph - BSTX (65):
[0061] Suitable examples of signal sequences can be derived from yeasts in general or any of the following specific genera of fungi: Aspergillus, Trichoderma, Chrysosporium, Pichia, Neurospora, Rhizomucor, Hansenula, Humicola, Mucor, Tolypocladium, Fusarium, Penicillium, Saccharomyces, Talaromyces or alternative sexual forms thereof like Emericella, Hypocrea. Signal sequences that are particularly useful are often natively associated with the following proteins a cellobiohydrolase, an endoglucanase, a beta-galactosidase,a xylanase, a pectinase, an esterase, a hydrophobin, a protease or an amylase. Examples include amylase or glucoamylase of Aspergillus or Humicola (4), TAKA amylase of Aspergillus oryzae, alpha-amylase of Aspergillus niger, carboxyl peptidase of Mucor (U.S. Pat. No. 5,578,463), a lipase or proteinase from Rhizomucor miehei, cellobiohydrolase of Trichoderma (5), beta-galactosidaseof Penicillium canescens and alpha mating factor of Saccharomyces.

PGPUB-DOCUMENT-NUMBER: 20030162218

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030162218 A1

TITLE: High-throughput screening of expressed DNA libraries in filamentous fungi

PUBLICATION-DATE: August 28, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Emalfarb, Mark A.	Jupiter	FL	US	
Punt, Peter J.	Houten		NL	
Zeijl, Cornelia van	EP Vleuten-de-Meern		NL	
Hondel, Cornelius van den	Gouda		NL	

APPL-NO: 09/ 834434

DATE FILED: April 13, 2001

RELATED-US-APPL-DATA:

child 09834434 A1 20010413

parent continuation-in-part-of PCT/US00/10199 20000413 US UNKNOWN

US-CL-CURRENT: 435/7.1, 435/254.2 , 435/254.3 , 435/320.1 , 435/483 , 435/69.1 , 536/23.2

ABSTRACT:

The invention provides a method for the expression of exogenous DNA libraries in filamentous fungi. The fungi are capable of processing intron-containing eukaryotic genes, and also can carry out post-translational processing steps such as glyclosylation and protein folding. The invention provides for the use of fungi with altered morphology, which permits high-throughput screening and directed molecular evolution of expressed proteins. The same transformed fungi may be used to produce larger quantities of protein for isolation, characterization, and application testing, and may be suitable for commercial production of the protein as well.

---------- KWIC ---------

Detail Description Paragraph - DETX (17):

[0081] An expression-regulating region comprises a promoter sequence operably linked to a nucleic acid sequence encoding the protein to be expressed. The promoter is linked such that the positioning vis--vis the initiation codon of the sequence to be expressed allows expression. The promoter sequence can be constitutive but preferably is inducible. Use of an inducible promoter and appropriate induction media favors expression of genes operably linked to the promoter. Any expression regulating sequence from a homologous species, or from a heterologous strain capable of permitting expression of a protein, is envisaged. The expression regulating sequence is suitably a fungal expression-regulating region, e.g. an ascomycete regulating region. Suitably the ascomycete expression regulating region is a regulating

region from any of the following genera: Aspergillus, Trichoderma, Chrysosporium, Humicola, Neurospora, Tolypocladium, Fusarium, Penicillium, Talaromyces, or alternative sexual forms thereof such as Emericela and Hypocrea. The cellobiohydrolase promoter from Trichoderma; alcohol dehydrogenase A, alcohol debydrogenase R, glutamate dehydrogenase, TAKA amylase, glucoamylase, and glyceraldehyde phosphate dehydrogenase promoters from Aspergillus; phosphoglycerate and cross-pathway control promoters of Neurospora; lipase and aspartic proteinase promoter of Rhizomucor miehei; beta-galactosidase promoter of Penicillium canescens; and cellobiohydrolase, endoglucanase, xylanase, glyceraldehyde-3-phosphate dehydrogenase A, and protease promoters from Chrysosporium are representative examples. An expression regulating sequence from the same genus as the host strain is preferable, as it is more likely to be specifically adapted to the host.

Detail Description Paragraph - DETX (30):

[0094] Any signal sequence capable of permitting secretion of a protein from a Chrysosporium strain is envisaged. Such a signal sequence is preferably a fungal signal sequence, more preferably an Ascomycete signal sequence. Suitable signal sequences can be derived from eukaryotes generally, preferably from yeasts or from any of the following genera of fungi: Aspergillus, Trichoderma, Chrysosporium, Pichia, Neurospora, Rhizomucor, Hansenula, Humicola, Mucor, Tolypocladium, Fusarium, Penicillium, Saccharomyces, Talaromyces or alternative sexual forms thereof such as Emericella and Hypocrea. Signal sequences that are particularly useful are those natively associated with cellobiohydrolase, endoglucanase, beta-galactosidase, xylanase, pectinase, esterase, hydrophobin, protease or amylase. Examples include amylase or glucoamylase of Aspergillus or Humicola, TAKA amylase of Aspergillus oryzae, .alpha.-amylase of Aspergillus niger, carboxyl peptidase of Mucor (U.S. Pat. No. 5,578,463), a lipase or proteinase from Rhizomucor miehei, cellobiohydrolase of Trichoderma, beta-galactosidase of Penicillium canescens CBH1 from Chrysosporium, and the alpha mating factor of Saccharomyces.

PGPUB-DOCUMENT-NUMBER: 20030082595

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030082595 A1

TITLE: Nucleic acids of aspergillus fumigatus encoding industrial enzymes and methods of use

PUBLICATION-DATE: May 1, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Jiang, Bo	Montreal		CA	
Storms, Reginald	Beaconsfield		CA	
Roemer, Terry	Montreal		CA	
Bussey, Howard	Westmount		CA	

APPL-NO: 10/ 213990

DATE FILED: August 5, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60309870 20010803 US

US-CL-CURRENT: 435/6, 435/101 , 435/189 , 435/200 , 435/320.1 , 435/325 , 435/69.1 , 536/123 , 536/23.2

ABSTRACT:

The present invention provides nucleotide sequences of Aspegillus fumigatus that encode proteins which exhibit enzyme activities. Vectors, expression constructs, and host cells comprising the nucleotide sequences of the enzyme genes are also provided. The invention further provides methods for producing the enzymes, and methods for modifying the enzymes in order to improve their desirable characteristics. The activities displayed by the enzymes of the invention include those of a tannase, cellulase, glucose oxidase, glucoamylase, phytase, .beta.-galactosidases, invertase, lipase, .alpha.-amylase, laccase, polygalacturonase or xylanase. The enzymes of the invention can be used in a variety of industrial processes. Enzymatically active compositions in various forms as well as antibodies to the enzymes and fragments thereof, are also provided.

---------- KWIC ---------

Detail Description Paragraph - DETX (48):

[0061] In addition, the genuses of isolated nucleic acid molecules provided in various embodiments of the invention does not comprise the nucleotide sequence of Genbank Accession No. D63338 encoding a tannase of Aspergillus oryzae, Genbank Accession No. AB022429 encoding a cellobiohydrolase II of Acremonium celluloticus Y-94, Genbank Accession No. AE004826 encoding an enzyme of Pseudomonas aeruginosa, Genbank Accession No. U56240 encoding a glucose oxidase of Talaromyces flavus, Genbank Accession No. AF012277 encoding a glucose oxidase of Penicillium amagasakiense, Genbank Accession No. U59804 encoding a phytase of Aspergillus fumigatus, Genbank Accession No. S37150 encoding a beta-galactosidase of Aspergillus niger, Genbank Accession No.

A00968 encoding a beta-galactosidase of Aspergillus niger, Genbank Accession No. AJ304803 encoding a glucoamylase of Talaromyces emersonii, Genbank Accession No. AJ289046 encoding a fructosyltransferase of Aspergillus sydowii, Genbank Accession No. A84689 encoding a protein product of Aspergillus tubingensis, Genbank Accession No. X12726 encoding an alpha-pre-amylase of Aspergillus oryzae, Genbank Accession No. AB008370 encoding an acid-stable alpha-amylase of Aspergillus kawachii, Genbank Accession No. AF208225 encoding an alpha-anylase Amy A of Aspergillus nidulans, Genbank Accession No. AF104823 of a gene product of Aspergillus fumigatus, Genbank Accession No. 010460 encoding a glucoarnylase Aspergillus shirousami, Genbank Accession No. AF052061 encoding a polygalacturonase of Ophiostoma novo-ulmi, Genbank Accession No. X58892 encoding a polygalacturonase of Aspergillus niger, Genbank Accession No. Y18805 encoding an endo-polygalacturonase B of Aspergillus niger, Genbank Accession No. AB003085 encoding XynG1 of Aspergillus oryzae, Genbank Accession No. AB044941 encoding a xylanase G2 of Aspergillus oryzae, and Genbank Accession No. AB035540 encoding a xylanase A of Penicillium sp.40. The gene products encoded by the foregoing Genbank nucleotide sequences are also not included in the genuses of gene products contemplated in the present invention.

PGPUB-DOCUMENT-NUMBER: 20030059901

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030059901 A1

TITLE: Process for isomaltose production

PUBLICATION-DATE: March 27, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Pedersen, Sven	Gentofte		DK	
Hendriksen, Hanne Vang	Holte		DK	

APPL-NO: 09/ 976224

DATE FILED: October 12, 2001

RELATED-US-APPL-DATA:

child 09976224 A1 20011012

parent division-of 09200109 19981125 US GRANTED

parent-patent 6329182 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	1997 01356	1997DK-1997 01356	November 26, 1997

US-CL-CURRENT: 435/101, 435/100

ABSTRACT:

The present invention relates to a method of producing oligosaccharide syrups, in particular to the production of syrups having a high concentration of saccharides with a degree of polymerization of at least 2, comprising the steps of: enzymatic reaction of a substrate at a temperature in the range of 50.degree. C. to 100.degree. C. obtaining a saccharide solution comprising monosaccharides and disaccharides, trisaccharides and higher saccharides; nanofiltration of the saccharide solution at a temperature in the range of 60.degree. C. to 100.degree.C. obtaining a syrup essentially comprising disaccharides, trisaccharides and higher saccharides; recovering said syrup; optionally recycling the permeate resulting from the nanofiltration step to the enzymatic reaction.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a division of U.S. patent application Ser. No. 09/200,109, filed on Nov. 25, 1998, now allowed, and claims priority from Danish application no. 1356/97, filed on Nov. 27, 1997, the contents of which are fully incorporated herein by reference.

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Detail Description Paragraph - DETX (20):

[0053] In a preferred embodiment of the present invention heatstable glucoamylase enzymes are utilised for the enzymatic reaction. The glucoamylase enzyme may preferably be derived from a strain of Aspergillus, in particular Aspergillus niger, a strain of Clostridium, in particular Clostridium thermoamylolyticum, Clostridium thermosulphurogenes, Clostridium thermohydrosulphuricum, a strain of Pestalotiopsis, or a strain of Talaromyces, in particular Talaromyces duponti, Talaromyces emersonii and Talaromyces thermophilus.

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TITLE: Glucoamylase variants

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INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Nielsen, Bjarne Roenfeldt	Virum		DK	
Svendsen, Allan	Birkeroed		DK	
Pedersen, Henrik	Odense SV		DK	
Vind, Jesper	Lyngby		DK	
Hendriksen, Hanne Vang	Holte		DK	
Frandsen, Torben Peter	Frederiksberg C		DK	

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RELATED-US-APPL-DATA:

child 10038723 A1 20020102

parent division-of 09351814 19990712 US GRANTED

parent-patent 6352851 US

non-provisional-of-provisional 60093528 19980721 US

non-provisional-of-provisional 60115545 19990112 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	PA 1998 00937	1998DK-PA 1998 00937	July 15, 1998
DK	PA 1998 01667	1998DK-PA 1998 01667	December 17, 1998

US-CL-CURRENT: 435/202, 435/204 , 435/320.1 , 435/325 , 435/69.1 , 536/23.2

ABSTRACT:

The invention relates to a variant of a parent fungal glucoamylase, which exhibits improved thermal stability and/or increased specific activity using saccharide substrates.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a division of U.S. patent application Ser. No. 09/351,814, filed on Jul. 12, 2001, and claims priority under 35 U.S.C. 119 of Danish application nos. PA 1998 00937 and PA 19980167 filed on Jul. 15, 1998 and Dec. 17, 1998, respectively, and U.S provisional application Nos. 60/093,528 and 60/115,545 filed on Jul. 21, 1998 and Jan. 12, 1999, respectively, the contents of which are fully incorporated herein by reference.

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Summary of Invention Paragraph - BSTX (11):
[0009] The object of the present invention is to provide improved glucoamylase variants with improved thermostability and/or increased specific activity suitable for use in, e.g., the saccharification step in starch conversion processes.

Summary of Invention Paragraph - BSTX (12):
[0010] The term "a glucoamylase variant with improved thermostability" means in the context of the present invention a glucoamylase variant which has a higher T.sub.1/2 (half-time) than the corresponding parent glucoamylase. The determination of T.sub.1/2 (Method I and Method II) is described below in the "Materials & Methods" section.

Summary of Invention Paragraph - BSTX (13):
[0011] The term "a glucoamylase variant with increased specific activity" means in the context of the present invention a glucoamylase variant with increased specific activity towards the .sub..alpha.-1,4 linkages in the saccharide in question. The specific activity is determined as k.sub.cat or AGU/mg (measured as described below in the "Materials & Methods" section). An increased specific activity means that the k.sub.cat or AGU/mg values are higher when compared to the k.sub.cat or AGU/mg values, respectively, of the corresponding parent glucoamylase.

Summary of Invention Paragraph - BSTX (14):
[0012] The inventors of the present invention have provided a number of improved variants of a parent glucoamylase with improved thermostability and/or increased specific activity in comparison to the parent corresponding enzyme. The improved thermal stability is obtained by substituting selected positions in a parent glucoamylase. This will be described in details below.

Detail Description Paragraph - DETX (2):
[0035] A goal of the work underlying the present invention was to improve the thermal stability and/or increase the specific activity of particular glucoamylases which are obtainable from fungal organisms, in particular strains of the Aspergillus genus and which themselves had been selected on the basis of their suitable properties in starch conversion or alcohol fermentation.

Detail Description Paragraph - DETX (3):
[0036] Identifying Positions and/or Regions to be Mutated to Obtain Improved Thermostability and/or Increased Specific Activity

Detail Description Paragraph - DETX (15):
[0048] Regions found to be of interest for increasing the specific activity and/or improved thermostability are the regions in proximity to the active site. Regions positioned in between the .sub..alpha.-helixes, and which may include positions on each side of the N- and C-terminal of the .sub..alpha.-helixes, at the substrate binding site is of importance for the activity of the enzyme. These regions constitute the following regions:

Detail Description Paragraph - DETX (26):
[0059] The present inventors have found that it is in fact possible to improve the thermal stability and/or to increase the specific activity of a parent glucoamylase by modification of one or more amino acid residues of the amino acid sequence of the parent glucoamylase. The present invention is based on this finding.

Detail Description Paragraph - DETX (29):

[0062] Parent glucoamylase contemplated according to the present invention include fungal glucoamylases, in particular fungal glucoamylases obtainable from an Aspergillus strain, such as an Aspergillus niger or Aspergillus awamori glucoamylases and variants or mutants thereof, homologous glucoamylases, and further glucoamylases being structurally and/or functionally similar to SEQ ID NO: 2. Specifically contemplated are the Aspergillus niger glucoamylases G1 and G2 disclosed in Boel et al. (1984), "Glucoamylases G1 and G2 from Aspergillus niger are synthesized from two different but closely related mRNAs", EMBO J. 3 (5), p. 1097-1102,. The G2 glucoamylase is disclosed in SEQ ID NO: 2. The G1 glucoamylase is disclosed in SEQ ID NO: 13. Another AMG backbone contemplated is Talaromyces emersonii, especially Talaromyces emersonii DSM disclosed in WO 99/28448 (Novo Nordisk).

Detail Description Paragraph - DETX (279):
[0312] As substrate binding may improve the stability region 93-127, Region: 170-184, Region: 305-319 are also contemplated for thermostabilization according to the present invention.

Detail Description Paragraph - DETX (444):
[0477] In a third aspect the invention relates to a variant of a parent glucoamylase with increased specific activity comprising one or more mutation(s) in the following position(s) or region(s) in the amino acid sequence shown in NO: 2:

Detail Description Paragraph - DETX (617):
[0650] By using a thermostable glucoamylase variant of the invention saccharification processes may be carried out at a higher temperature than traditional batch saccharification processes. According to the invention saccharification may be carried out at temperatures in the range from above 60-80.degree. C., preferably 63-75.degree. C. This apply both for traditional batch processes (described above) and for continuous saccharification processes.

Detail Description Paragraph - DETX (618):
[0651] Actually, continuous saccharification processes including one or more membrane separation steps, i.e. filtration steps, must be carried out at temperatures of above 60.degree. C. to be able to maintain a reasonably high flux over the membrane or to minimize microbial contamination. Therefore, the thermostable variants of the invention provides the possibility of carrying out large scale continuous saccharification processes at a fair price and/or at a lower enzyme protein dosage within a period of time acceptable for industrial saccharification processes. According to the invention the saccharification time may even be shortened.

Detail Description Paragraph - DETX (622):
[0655] A glucoamylase with an increased specific activity towards saccharides present in the solution after liquefaction and saccharides formed during saccharification would be an advantage as a reduced enzyme protein dosage or a shorter process time then could be used. In general, the glucoamylase has a preference for substrates consisting of longer saccharides compared to short chain saccharides and the specific activity towards e.g. maltoheptaose is therefore approximately 6 times higher than towards maltose. An increased specific activity towards short chain saccharides such as maltose (without reducing the activity towards oligosaccharides) would therefore also permit using a lower enzyme dosage and/or shorter process time.

Detail Description Paragraph - DETX (623):
[0656] Furthermore, a higher glucose yield can be obtained with a glucoamylase variant with an increased alpha-1,4 hydrolytic activity (if the

alpha-1,6 activity is unchanged or even decreased), since a reduced amount of enzyme protein is being used, and alpha-1,6 reversion product formation therefore is decreased (less isomaltose).

Detail Description Paragraph - DETX (629):
[0662] herein the enzymatic saccharification is carried out using a thermostable lucoamylase variant of the invention.

Detail Description Paragraph - DETX (697):
[0727] Screening for Thermostable AMG Variants

Detail Description Paragraph - DETX (698):
[0728] The libraries are screened in the thermostable filter assay described below.

Detail Description Paragraph - DETX (699):
[0729] Filter Assay for Thermostability

Detail Description Paragraph - DETX (731):
[0758] Construction, by Localized random, Doped Mutagenesis, of A niger AMG Variants Having Improved Thermostability Compared to the Parent Enzyme

Detail Description Paragraph - DETX (732):
[0759] To improve the thermostability of the A. niger AMG random mutagenesis in pre-selected region was performed.

Detail Description Paragraph - DETX (804):
[0831] The library was screened in the thermostability filter assays using a Protran filter and incubating at 67-69.degree. C. as described in the "Material & Methods" section above

Detail Description Paragraph - DETX (806):
[0832] Construction, by PCR Shuffling Spiked with DNA Oligos, of A. niger AMG Variants Having Improved Thermostability Compared to the Parent Enzyme

Detail Description Paragraph - DETX (954):
[0978] Thermostability at 70.degree. C.

Detail Description Paragraph - DETX (956):
[0980] The thermostability was determined as T.sub.1/2 using Method I, and as % residual activity after incubation for 30 minutes in 50 mM NaOAc, pH 4.5, 70.degree. C., 0.2 AGU/ml, as described in the "Material & Methods" section above. The result of the tests are listed in the Table below and compared to the wild-type A. niger AMG G2.

Detail Description Paragraph - DETX (958):
[0981] Thermostability at 68.degree. C.

Detail Description Paragraph - DETX (960):
[0983] The thermostability was determined as T1/2 using method I at 68.degree. C. as described in the "Materials & Methods" section and compared to the wild-type A. niger AMG G2 under the same conditions. Evaluation of variants were performed on culture broth after filtration of the supernatants.

Detail Description Paragraph - DETX (963):
[0985] Thermostability at 68.degree. C.

Detail Description Paragraph - DETX (965):
[0987] The thermostability was determined as T1/2 using method I at

68.degree. C. as described in the "Materials & Methods" section and compared to the wild-type A. niger AMG G2 under the same conditions. Evaluation of variants were performed on culture broth after filtration of the supernatants.

Detail Description Paragraph - DETX (967):
[0988] Thermostability at 68.degree. C.

Detail Description Paragraph - DETX (968):
[0989] AMG G2 variants were constructed using the approach described in Example 3. The thermostability was determined as T1/2 using method I at 68.degree. C. as described in the "Materials & Methods" section and compared to the wild-type A. niger AMG G2 under the same conditions. Evaluation of variants were performed on culture broth after filtration of the supernatants.

Detail Description Paragraph - DETX (970):
[0990] Thermostability at 70.degree. C.

Detail Description Paragraph - DETX (972):
[0992] The thermostability was determined as % residual activity using Method I in 50 mM NaOAc, pH, 4.5, 70.degree. C., as described in the "Material & Methods" section above. The result of the test is listed in the Table below and compared to the wild-type A. niger AMG G2.

Detail Description Paragraph - DETX (974):
[0993] thermostability at 70.degree. C. in presence of 30% glucose

Detail Description Paragraph - DETX (976):
[0995] The thermostability was determined as T1/2 using method II at 70.degree. C. as described in the "Materials & Methods" section and compared to the wild-type A. niger AMG G2 under the same conditions.

Detail Description Paragraph - DETX (979):
[0997] Saccharification performance of the AMG variants S119P+Y402F+S411V and PLASD(N-terminal)+V59A+A393R+T490A, respectively, both having improved thermostability are tested at 70.degree. C. as described below.

Detail Description Table CWU - DETL (9):
9 Thermostability at 70.degree. C. on purified samples. Enzyme T1/2 (min) 15 AMG G2 (wild type) 7.4 16 T2E + T379A + S386K + A393R 11.6 17 E408R + S386N 10.2 18 T2Q + A11P + S394R 9.8 19 A1V + L66R + Y402F + N427S + S486G 14.1 20 A393R 14.6 21 T2R + S386R + A393R 14.1 22 A393R + L410R 12.9 23 Y402F 10.1

Claims Text - CLTX (3):
3. A variant of a parent glucoamylase with improved thermostability comprising one or more mutation(s) in the following position(s) or region(s) in the amino acid sequence shown in NO: 2: Region: 1-18, Region: 19-35, Region: 73-80, Region: 93-127, Region: 170-184, Region: 200-212, Region: 234-246, Region: 287-319 Region: 334-341, Region: 353-374, Region: 388-414. Region: 445-470, and/or in a corresponding position or region in a homologous glucoamylase which displays at least 60% homology with the amino acid sequences shown in SEQ ID NO: 2, with the exception of the following substitutions: N20C, A27C, S30P, A246C.

Claims Text - CLTX (4):
4. A variant of a parent glucoamylase with increased specific activity comprising one or more mutation(s) in the following position(s) or region(s) in the amino acid sequence shown in NO: 2: Region: 1-18, Region: 40-62, Region: 93-127, Region: 170-184, Region: 200-212, Region: 234-246, Region: 287-319,

Region: 388-414, and/or in a corresponding position or region in a homologous glucoamylase which displays at least 60% homology with the amino acid sequences shown in SEQ ID NO: 2, with the exception of the following substitutions: S411G.

Claims Text - CLTX (27):

27. A method for improving the thermostability and/or of increasing the specific activity of a parent glucoamylase by making a mutation in one or more of the following position(s) or region(s) in the amino acid sequence shown in NO: 2: Region: 1-18, Region: 19-35, Region: 40-62, Region: 73-80, Region: 93-127, Region: 170-184, Region: 200-212, Region: 234-246, Region: 287-319, Region: 334-341, Region: 353-374, Region: 388-414, Region: 445-470, and/or in a corresponding position or region in a homologous glucoamylase which displays at least 60% homology with the amino acid sequences shown in SEQ ID NO: 2.

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TITLE: Thermostable glucoamylase

PUBLICATION-DATE: February 6, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Nielsen, Bjarne Ronfeldt	Virum		DK	
Nielsen, Ruby Ilum	Farum		DK	
Lehmbeck, Jan	Vekso		DK	

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DATE FILED: March 29, 2001

RELATED-US-APPL-DATA:

child 09821616 A1 20010329

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parent-patent 6255084 US

child 09199290 19981124 US

parent continuation-in-part-of 08979673 19971126 US ABANDONED

child 09199290 19981124 US

parent continuation-in-part-of 09107657 19980630 US ABANDONED

non-provisional-of-provisional 60070746 19980108 US

non-provisional-of-provisional 60094344 19980728 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	1557/97	1997DK-1557/97	December 30, 1997
DK	PA 1998 00925	1998DK-PA 1998 00925	July 10, 1998

US-CL-CURRENT: 435/96, 536/23.2

ABSTRACT:

The invention relates to an isolated thermostable glucoamylase derived from Talaromyces emersonii suitable for starch conversion processes.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of application Ser. No. 09/199,290 filed Nov. 24, 1998, which is a continuation-in-part of application Ser. Nos. 08/979,673 and 09/107,657 filed Nov. 26, 1997 and Jun. 30, 1998, respectively, and claims priority under 35 U.S.C. 119 of Danish application

nos. 1557/97 and PA 1998 00925 filed Dec. 30, 1997 and Jul. 10, 1998, respectively, and U.S. application Ser. Nos. 60/070,746 and 60/094,344 filed Jan. 8, 1998 and Jul. 28, 1998, respectively, the contents of which are fully incorporated herein by reference.

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Abstract Paragraph - ABTX (1):
The invention relates to an isolated thermostable glucoamylase derived from Talaromyces emersonii suitable for starch conversion processes.

Title - TTL (1):
Thermostable glucoamylase

Summary of Invention Paragraph - BSTX (2):
[0002] The present invention relates to a thermostable glucoamylase suitable for, e.g., starch conversion, e.g., for producing glucose from starch. The present invention also relates to the use of said thermostable glucoamylase in various processes, in particular in the saccharification step in starch convention processes.

Summary of Invention Paragraph - BSTX (8):
[0007] U.S. Patent No. 4,247,637 describes a thermostable glucoamylase having a molecular weight of about 31,000 Da derived from Talaromyces duponti suitable for saccharifying a liquefied starch solution to a syrup. The glucoamylase is stated to retain at least about 90% of its initial glucoamylase activity when held at 70.degree. C. for 10 minutes at pH 4.5.

Summary of Invention Paragraph - BSTX (9):
[0008] U.S. Pat. No. 4,587,215 discloses a thermostable amyloglucosidase derived from the species Talaromyces thermophilus with a molecular weight of about 45,000 Da. The disclosed amyloglucosidase (or glucoamylase) loses its enzymatic activity in two distinct phases, an initial period of rapid decay followed by a period of slow decay. At 70.degree. C. (pH=5.0) the half-life for the fast decay is about 18 minutes with no measurable loss of activity within an hour in the second phase of decay.

Summary of Invention Paragraph - BSTX (12):
[0010] The present invention is based upon the finding of a novel thermostable glucoamylase suitable for use, e.g., in the saccharification step in starch conversion processes.

Summary of Invention Paragraph - BSTX (15):
[0013] The inventors of the present invention have isolated, purified and characterized a thermostable glucoamylase from a strain of Talaromyces emersonii now deposited with the Centraalbureau voor Schimmelcultures under the number CBS 793.97.

Summary of Invention Paragraph - BSTX (20):
[0018] The isolated glucoamylase has a very high thermal stability in comparison to prior art glucoamylases, such as the Aspergillus niger glucoamylase (available from Novo Nordisk A/S under the trade name AMG). The T1/2 (half-life) was determined to be about 120 minutes at 700C (pH 4.5) as described in Example 2 below. The T1/2 of the recombinant T. emersonii AMG expressed in yeast was determined to be about 110 minutes as described in Example 12.

Summary of Invention Paragraph - BSTX (29):

[0027] Finally, the invention relates to an isolated pure culture of the microorganism Talaromyces emersonii CBS 793.97 or a mutant thereof capable of producing a glucoamylase of the invention.

Brief Description of Drawings Paragraph - DRTX (2):

[0028] FIG. 1 shows the SDS-PAGE gel (stained with Coomassie Blue) used for determining the molecular weight (M.sub.w) of the purified Talaromyces emersonii CBS 793.97 glucoamylase of the present invention.

Brief Description of Drawings Paragraph - DRTX (6):

[0032] FIG. 2 shows the pH activity profile of Talaromyces emersonii and Aspergillus niger glucoamylase (AMG) in 0.5% maltose at 60.degree. C.;

Brief Description of Drawings Paragraph - DRTX (7):

[0033] FIG. 3 shows the temperature activity profile of the Talaromyces emersonii CBS 793.97 glucoamylase vs. Aspergillus niger glucoamylase (AMG)

Brief Description of Drawings Paragraph - DRTX (8):

[0034] FIG. 4 shows the curve for determining T.sub.1/2 (half-life) in 50 mM NaOAc, 0.2 AGU/ml, pH 4.5, at 70.degree. C. of Talaromyces emersonii CBS 793.97 glucoamylase vs. Aspergillus niger glucoamylase (AMG);

Brief Description of Drawings Paragraph - DRTX (15):

[0041] FIG. 10 shows the SDS page gel of two transformants, JaL228#5.77 and HowB112#8.10, expressing the Talaromyces emersonii glucoamylase of the invention. JaL228 and HowB112 are the untransformed parent strains. MW: Promega's Protein Molecular;

Brief Description of Drawings Paragraph - DRTX (18):

[0044] FIG. 13 shows the result of the test for determining the thermostability of recombinant Talaromyces emersonii AMG produced in yeast at 70.degree. C., pH 4.5, 0.2 AGU/ml. T.sub.1/2 was determined to about 110.degree. C.

Detail Description Paragraph - DETX (2):

[0045] The present invention is based upon the finding of a novel thermostable glucoamylase suitable for use in, e.g., the saccharification step in a starch conversion process.

Detail Description Paragraph - DETX (3):

[0046] The inventors of the present invention have isolated, purified and characterized a glucoamylase from a strain of Talaromyces emersonii CBS 793.97. The glucoamylase turned out to have a very high thermal stability in comparison to prior art glucoamylases.

Detail Description Paragraph - DETX (5):

[0048] T.sub.1/2 (half-life) of the isolated Talaromyces emersonii CBS 793.97 glucoamylase was determined to be about 120 minutes at 70.degree. C. as described in Example 2 below and to be about 110.degree. C. for the T. emersonii produced in yeast as described in Example 12.

Detail Description Paragraph - DETX (15):

[0058] Talaromyces emersonii Glucoamylase Amino Acid Sequence

Detail Description Paragraph - DETX (16):
[0059] The inventors have sequenced the thermostable glucoamylase derived from Talaromyces emersonii CBS 793.97 as will be described further in the Example 3 below. According to the invention the Talaromyces AMG may have a Asp145Asn (or D145N) substitution (using SEQ ID NO: 7 numbering).

Detail Description Paragraph - DETX (37):
[0080] The present invention provides a method of using the thermostable glucoamylase of the invention for producing glucose and the like from starch. Generally, the method includes the steps of partially hydrolyzing precursor starch in the presence of .alpha.-amylase and then further hydrolyzing the release of D-glucose from the non-reducing ends of the starch or related oligo- and polysaccharide molecules in the presence of glucoamylase by cleaving .alpha.-(1.degree.4) and .alpha.-(1.degree.6) glucosidic bonds.

Detail Description Paragraph - DETX (40):
[0083] By using a thermostable glucoamylase of the invention saccharification processes may be carried out at a higher temperature than traditional batch saccharification processes. According to the invention saccharification may be carried out at temperatures in the range from above 60-80.degree. C., preferably 63-75.degree. C. This applies both for traditional batch processes (described above) and for continuous saccharification processes.

Detail Description Paragraph - DETX (41):
[0084] Actually, continuous saccharification processes including one or more membrane separation steps, i.e., filtration steps, must be carried out at temperatures of above 60.degree. C. to be able to maintain a reasonably high flux over the membrane. Therefore, a thermostable glucoamylase of the invention provides the possibility of carrying out large scale continuous saccharification processes at a fair price within and period of time acceptable for industrial saccharification processes. According to the invention the saccharification time may even be shortened.

Detail Description Paragraph - DETX (42):
[0085] The activity of a glucoamylase of the invention is generally substantially higher at temperatures between 60.degree. C.-80.degree. C. than at the traditionally used temperature between 30-60.degree. C. Therefore, by increasing the temperature at which the glucoamylase operates the saccharification process may be carried out within a shorter period of time or the process may be carried out using lower enzyme dosage.

Detail Description Paragraph - DETX (44):
[0087] By using a glucoamylase with increased specific activity (measured as activity towards maltose), a lower enzyme dosage may be required in the saccharification process.

Detail Description Paragraph - DETX (55):
[0098] Glucoamylase derived from the deposited filamentous fungus Talaromyces emersonii CBS No. 793.97 has been deposited with the Centraalbureau voor Schimmelcultures, P.O. Box 273, 3740 AG Baarn, the Netherlands, for the purposes of patent procedure on the date indicated below. CBS being an international depository under the Budapest Treaty affords permanence of the deposit in accordance with rule 9 of said treaty.

Detail Description Paragraph - DETX (66):
[0109] T. emersonii glucoamylase gene with introns is shown in FIG. 5 and SEQ ID NO: 33. The introns are shown in FIG. 5.

Detail Description Paragraph - DETX (126):
[0166] Characterisation of the Talaromyces emersonii Glucoamylase

Detail Description Paragraph - DETX (127):
[0167] The purified Talaromyces emersonii CBS 793.97 glucoamylase was used for characterisation.

Detail Description Paragraph - DETX (133):
[0173] The pH-activity dependency of the Talaromyces emersonii glucoamylase was determined and compared with profile of Aspergillus niger glucoamylase.

Detail Description Paragraph - DETX (136):
[0176] The temperature-activity dependency of the Talaromyces emersonii glucoamylase of the invention was determined and compared with the profile of Aspergillus niger glucoamylase.

Detail Description Paragraph - DETX (139):
[0179] The thermal stability of the Talaromyces emersonii glucoamylase was determined and compared with the thermal stability of Aspergillus niger glucoamylase.

Detail Description Paragraph - DETX (141):
[0181] The T1/2 of the Talaromyces emersonii glucoamylase was determined to about 120 minutes at 70.degree. C. The T1/2 of the Aspergillus niger glucoamylase was determined to 7 minutes under the same conditions (See FIG. 4).

Detail Description Paragraph - DETX (145):
[0184] Sequencing of the N-terminal of T. emersonii Glucoamylase

Detail Description Paragraph - DETX (146):
[0185] The N-terminal amino acid sequence of T. emersonii glucoamylase was determined following SDS-PAGE and electroblotting onto a PVDF-membrane. Peptides were derived from reduced and S-carboxymethylated glucoamylase by cleaving with a lysyl-specific protease. The resulting peptides were fractionated and re-purified using RP-HPLC before subjected to N-terminal sequence determination.

Detail Description Paragraph - DETX (148):
[0186] The Full Length T. emersonii Glucoamylase

Detail Description Paragraph - DETX (149):
[0187] The full length T. emersonii glucoamylase amino acid sequence shown in SEQ ID NO: 7 was identified using standard methods.

Detail Description Paragraph - DETX (151):
[0188] Cloning and Sequencing of the Talaromyces emersonii Glucoamylase Gene

Detail Description Paragraph - DETX (201):
[0235] Expression of Talaromyces emersonii Glucoamylase in Yeast

Detail Description Paragraph - DETX (204):
[0238] The yeast cells were grown at 30.degree. C. for 3 days in Sc-ura medium followed by growth for 3 days in YPD. The culture was then centrifuged and the supernatant was used for the thermostability assay described in the "Materials and Method" section.

Detail Description Paragraph - DETX (209):

[0242] 200 ml culture broth from fermentation of A. niger HowBll2 harboring the Talaromyces emersonii gene was centrifuged at 9000 rpm and dialyzed against 20 mM NaOac, pH 5 over night. The solution was then applied on a S Sepharose column (200 ml) previously equilibrated in 20 mM NaOAc, pH 5. The glucoamylase was collected in the effluent, and applied on a Q Sepharose column (50 ml) previously equilibrated in 20 mM NaOAC, pH 4.5. Unbound material was washed of the column and the glucoamylase was eluted using a linear gradient from 0-0.3 M NaCl in 20 mM NaOAc over 10 column volumes. Purity of the glucoamylase fraction was checked by SDS-PAGE and only one single band was seen. The molecular weight was again found to about 70 kdal as seen for the wild type glucoamylase. The specific activity towards maltose was measured and a specific activity of 8.0 AGU/mg (37.degree. C.) and 21.0 AGU/mg (60.degree. C.) were found which is in accordance the data on the wild type enzyme.

Detail Description Paragraph - DETX (215):
[0246] The saccharification performance of the Talaromyces emersonii glucoamylase was tested at different temperatures with and without the addition of acid .alpha.-amylase and pullulanase. Saccharification was run under the following conditions:

Detail Description Paragraph - DETX (217):
[0248] Recombinant Talaromyces emersonii glucoamylase produced in A. niger: 0.24 or 0.32 AGU/g DS

Detail Description Paragraph - DETX (227):
[0257] The thermal stability of recombinant Talaromyces emersonii glucoamylase expressed in yeast (purified using the method described in Example 9) was determined at 70.degree. C., pH 4.5, 0.2 AGU/ml using the method described above in the "Material and Methods" section as "Thermal Stability I (T1/2 (half-life) determination of AMG".

Detail Description Paragraph - DETX (228):
[0258] FIG. 13 shows the result of the test. The T1/2 of the recombinant Talaromyces emersonii glucoamylase expressed in yeast was determined to about 110 minutes at 70.degree. C.

Claims Text - CLTX (1):
1. An isolated DNA sequence encoding an enzyme with glucoamylase activity, wherein the enzyme (a) is derived from Talaromyces and has a T1/2 (half-life) of at least 100 minutes in 50 mM NaOAc, 0.2 Novo Amyloglucosidase Unit (AGU)/ml, pH 4.5, at 70.degree. C. or (b) has an amino acid sequence that has at least 80% identity with the glucoamylase of SEQ ID NO:7:

PGPUB-DOCUMENT-NUMBER: 20030022348

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030022348 A1

TITLE: Modified forms of pullulanase

PUBLICATION-DATE: January 30, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Miller, Brian S.	Burlingame	CA	US	
Shetty, Jayarama K.	Pleasanton	CA	US	

APPL-NO: 10/ 245803

DATE FILED: September 16, 2002

RELATED-US-APPL-DATA:

child 10245803 A1 20020916

parent division-of 09262126 19990303 US PENDING

child 09262126 19990303 US

parent continuation-in-part-of 09034630 19980304 US ABANDONED

US-CL-CURRENT: 435/210, 435/252.3 , 435/252.31 , 435/320.1 , 435/69.1 , 536/23.2

ABSTRACT:

The present invention relates to modified pullulanases useful in the starch industry. The present invention provides methods for producing the modified pullulanase, enzymatic compositions comprising the modified pullulanase, and methods for the saccharification of starch comprising the use of the enzymatic compositions.

RELATED APPLICATIONS

[0001] The present application is a continuation-in-part application of U.S. application Ser. No. 09/034,630 filed Mar. 4, 1998 which is incorporated herein in its entirety.

---------- KWIC ---------

Detail Description Paragraph - DETX (88):

[0106] Example I illustrates the production of a modified pullulanase as described herein. The nucleic acid sequence encoding a pullulanase is modified by recombinant DNA techniques such as standard PCR primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. (Saiki, R. K., et al., 1988, Science 239:487-491.) and PCR fusion techniques (Fleming, A. B., et al. Appl. Environ. Microbiol 61, 3775-3780). DNA encoding the desired modified pullulanase is fused to the C-terminus of a signal sequence, preferably a host microorganism signal sequence. This construct is cloned and

transformed into a host cell, such as, B. subtilis or B. licheniformis, and cultured under standard fermentation conditions. The modified pullulanase is purified from the fermentation broth and assayed for activity.

Detail Description Paragraph - DETX (92):

[0108] Example III describes the saccharification process comparing enzymatic compositions comprising different percentages of pullulanase. Enzymatic compositions comprising either 20% glucoamylase:80% modified pullulanase (20:80) activity or 75% glucoamylase:25% pullulanase activity (75:25) were tested in saccharification processes at a concentration of 0.550, 0.635 and 0.718 liters of enzymatic composition per metric ton of dissolved solids. As shown in FIGS. 5A-5C, an enzymatic composition comprising 20% glucoamylase and 80% pullulanase activity is able to increase the final glucose yield without an increase in undesirable disaccharide formation. Furthermore, the absolute concentration of the 20:80 enzyme composition can be increased without the undesirable increase in disaccharide formation that is seen with the 75:25 enzyme composition or glucoamylase alone.

PGPUB-DOCUMENT-NUMBER: 20030013180

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030013180 A1

TITLE: MODIFIED FORMS OF PULLULANASE

PUBLICATION-DATE: January 16, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
MILLER, BRIAN S.	BURLINGAME	CA	US	
SHETTY, JAYARAMA K.	PLEASANTON	CA	US	

APPL-NO: 09/ 262126

DATE FILED: March 3, 1999

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

RELATED-US-APPL-DATA:

child 09262126 A1 19990303

parent continuation-in-part-of 09034630 19980304 US ABANDONED

US-CL-CURRENT: 435/210, 435/320.1 , 435/325 , 435/69.1 , 536/23.2

ABSTRACT:

The present invention relates to modified pullulanases useful in the starch industry. The present invention provides methods for producing the modified pullulanase, enzymatic compositions comprising the modified pullulanase, and methods for the saccharification of starch comprising the use of the enzymatic compositions.

RELATED APPLICATIONS

[0001] The present application is a continuation-in-part application of U.S. application Ser. No. 09/034,630 filed Mar. 4, 1998 which is incorporated herein in its entirety.

---------- KWIC ---------

Detail Description Paragraph - DETX (88):

[0106] Example I illustrates the production of a modified pullulanase as described herein. The nucleic acid sequence encoding a pullulanase is modified by recombinant DNA techniques such as standard PCR primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. (Saiki, R. K., et al., 1988, Science 239:487-491.) and PCR fusion techniques (Fleming, A. B., et al. Appl. Environ. Microbiol. 61, 3775-3780). DNA encoding the desired modified pullulanase is fused to the C-terminus of a signal sequence, preferably a host microorganism signal sequence. This construct is cloned and transformed into a host cell, such as, B. subtilis or B. licheniformis, and cultured under standard fermentation conditions. The modified pullulanase is

purified from the fermentation broth and assayed for activity.

Detail Description Paragraph - DETX (92):

[0108] Example III describes the saccharification process comparing enzymatic compositions comprising different percentages of pullulanase. Enzymatic compositions comprising either 20% glucoamylase:80% modified pullulanase (20:80) activity or 75% glucoamylase:25% pullulanase activity (75:25) were tested in saccharification processes at a concentration of 0.550, 0.635 and 0.718 liters of enzymatic composition per metric ton of dissolved solids. As shown in FIGS. 5A-5C, an enzymatic composition comprising 20% glucoamylase and 80% pullulanase activity is able to increase the final glucose yield without an increase in undesirable disaccharide formation. Furthermore, the absolute concentration of the 20:80 enzyme composition can be increased without the undesirable increase in disaccharide formation that is seen with the 75:25 enzyme composition or glucoamylase alone.

PGPUB-DOCUMENT-NUMBER: 20020187528

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020187528 A1

TITLE: Fermentation with a phytase

PUBLICATION-DATE: December 12, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Veit, Chris	Wake Forest	NC	US	
Felby, Claus	Herlev	NC	DK	
Peckous, Larry W.	Raleigh		US	
Olsen, Hans Sejr	Holte		DK	

APPL-NO: 10/ 164029

DATE FILED: June 6, 2002

RELATED-US-APPL-DATA:

child 10164029 A1 20020606

parent continuation-of 09788906 20010220 US PENDING

non-provisional-of-provisional 60185716 20000223 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	PA 2000 00281	2000DK-PA 2000 00281	February 23, 2000

US-CL-CURRENT: 435/105, 435/155 , 435/254.21

ABSTRACT:

The present invention relates to an improved fermenation process wherein phytic acid-containing material is fermented in the presence of a phytase.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. application Ser. No. 09/788,906, filed on Feb. 20, 2001, and claims the benefit of U.S. Provisional application No. 60/185,716, filed Feb. 23, 2000, and priority under 35 U.S.C. 119 of Danish application PA 2000 00281, filed Feb. 23, 2000, the contents of which are fully incorporated herein by reference.

---------- KWIC ---------

Detail Description Paragraph - DETX (71):

[0077] Other suitable Aspergillus glucoamylase variants include variants to enhance the thermal stability: G137A and G139A (Chen et al. (1996), Prot. Engng. 9, 499-505); D257E and D293E/Q (Chen et al. (1995), Prot. Engng. 8, 575-582); N182 (Chen et al. (1994), Biochem. J. 301, 275-281); disulphide bonds, A246C (Fierobe et al. (1996), Biochemistry, 35, 8698-8704; and introduction of Pro residues in position A435 and S436 (Li et al. (1997),

Protein Engng. 10, 1199-1204. Furthermore Clark Ford presented a paper on Oct. 17, 1997, ENZYME ENGINEERING 14, Beijing/China Oct. 12-17, 1997, Abstract number: Abstract book p. 0-61. The abstract suggests mutations in positions G137A, N20C/A27C, and S30P in an Aspergillus awamori glucoamylase to improve the thermal stability. Other glucoamylases include Talaromyces glucoamylases, preferably derived from Talaromyces emersonii (WO 99/28448), Talaromyces leycettanus (U.S. Pat. No. Re. 32,153), Talaromyces duponti, Talaromyces thermophilus (U.S. Pat. No. 4,587,215). Bacterial glucoamylases preferably include glucoamylases from the genus Clostridium, more preferably, C. thermoamylolyticum (EP 135,138), and C. thermohydrosulfuricum (WO 86/01831).

Detail Description Paragraph - DETX (84):

[0090] This aspect of the invention relates to a composition comprising a phytase and at least one carbohydrate-source generating enzyme (as defined above), preferably, a glucoamylase, such as an Aspergillus niger and/or Talaromuces emersonii glucoamylase. The composition may further comprise a protease, preferably, an acid protease, such as an acid fungal protease.

PGPUB-DOCUMENT-NUMBER: 20020164723

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020164723 A1

TITLE: Method of producing saccharide preparations

PUBLICATION-DATE: November 7, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Liaw, Gin C.	Decatur	IL	US	
Pedersen, Sven	Gentofte		DK	
Hendriksen, Hanne Vang	Holte		DK	
Svendsen, Allan	Birkerod		DK	
Nielsen, Bjarne Ronfeldt	Virum		DK	
Nielsen, Rudy Illum	Farum		DK	

APPL-NO: 09/ 908395

DATE FILED: July 18, 2001

RELATED-US-APPL-DATA:

child 09908395 A1 20010718

parent continuation-of 09632392 20000804 US GRANTED

parent-patent 6303346 US

child 09632392 20000804 US

parent continuation-of 09499531 20000210 US GRANTED

parent-patent 6136571 US

child 09499531 20000210 US

parent continuation-of 09198672 19981123 US GRANTED

parent-patent 6129788 US

child 09198672 19981123 US

parent continuation-in-part-of 09107657 19980630 US ABANDONED

child 09107657 19980630 US

parent continuation-in-part-of 08979673 19971126 US ABANDONED

US-CL-CURRENT: 435/96

ABSTRACT:

The present invention relates to a method for the production of saccharide preparations, i.e., syrups, by saccharifying a liquefied starch solution, which method comprises a saccharification step during which step one or more

enzymatic saccharification stages takes place, and the subsequent steps of one or more high temperature membrane separation steps, and re-circulation of the saccharification enzyme, in which method the membrane separation steps are carried out as an integral part of the saccharification step.
In another specific aspect, the invention provides a method of producing a saccharide preparation, which method comprises an enzymatic saccharification step, and the subsequent steps of one or more high temperature membrane separation steps and re-circulation of the saccharification enzyme.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. patent application Ser. No. 09/632,392, filed on Aug. 4, 2000, now allowed, which is a continuation of U.S. patent application Ser. No. 09/499,531, filed on Feb. 10, 2000, now U.S. Pat. No. 6,136,571, which is a continuation of U.S. patent application Ser. No. 09/198,672, filed on Nov. 23, 1998, now U.S. Pat. No. 6,129,788, which is a continuation-in-part of U.S. patent application Ser. No. 09/107,657, filed on Jun. 30, 1998, which is a continuation-in-part of U.S. patent application Ser. No. 08/979,673, filed on Nov. 26, 1997, the contents of which are fully incorporated herein by reference.

[0002] The present invention relates to the production of mono and/or oligosaccharides from starch, including dextrose, trehalose, isomaltooligosaccaharides, cyclodextrins and maltooligosaccharides. In a specific aspect, the invention provides a method of saccharifying a liquefied starch solution, which method comprises a saccharification step during which step one or more enzymatic saccharification stages takes place, and the subsequent steps of one or more high temperature membrane separation steps, and re-circulation of the saccharification enzyme, in which method the membrane separation steps are carried out as an integral part of the saccharification step.

[0003] In another specific aspect, the invention provides a method of producing a mono and/or oligosaccharide, such as dextrose, trehalose, isomaltooligosaccaharide, cyclodextrins and maltooligosaccharide preparation, which method comprises an enzymatic saccharification step, and the subsequent steps of one or more high temperature membrane separation steps and re-circulation of the saccharification enzyme.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (19):
[0021] Isomaltooligosaccharide syrups are sometimes referred to as "Alo mixtures" and defines a mixture containing isomaltose (O-.alpha.-D-glucopyranosyl-(1-6)-D-glucopyranose), panose, isomaltotriose and several other branched oligosaccharides composed of four and five glucose residues. "Alo mixture" syrups may be produced enzymatically from starch using a thermostable bacterial .alpha.-amylase in the liquefaction step. In the following step the liquefied starch is hydrolyzed or saccharified using a .beta.-amylase and a transglucosidase simultaneously.

Summary of Invention Paragraph - BSTX (40):
[0041] It has also been found that the efficiency of a nanofiltration step, when producing dextrose, can be further increased if the purification process is carried out at an elevated temperature (i.e., of above 63.degree. C.). Moreover, it has been found that the amount of by-products formed during reaction is decreased when a glucose solution (syrup) of a lower glucose content is applied to the membrane separation step. This allow for a more

efficient purification of the dextrose syrup, and dextrose preparations, of high purity can be more easily obtained. Finally, yields are improved and costs reduced by employing thermostable enzymes.

Detail Description Paragraph - DETX (55):
[0110] Isomaltooligosaccharide syryps or "Alo mixtures" may be produced from starch by first performing a liquefaction step using a thermostable bacterial .alpha.-amylase. The degree of hydrolysis (DE) of the starch is kept between 6-10. The liquefied starch is then subjected simultaneously to a .beta.-amylase (e.g., soybean .beta.-amylase) and a transglucosidase (e.g., from Aspergillus niger), 2-4 g and 0.2-0.3 g/kg starch, respectively, at 60.degree. C., pH 5.0, for about 72 hours. The reaction mixture is purified and concentrated to obtain the isomaltooligosaccharide product.

Detail Description Paragraph - DETX (75):
[0130] Thermostable Glucoamylase Enzymes

Detail Description Paragraph - DETX (76):
[0131] Preferably, the saccharification step of the invention is performed in presence of a thermostable glucoamylase enzyme (EC. 3.2.1.3).

Detail Description Paragraph - DETX (77):
[0132] Saccharification including using a thermostable AMG may be carried out as described in Example 3.

Detail Description Paragraph - DETX (79):
[0134] In another preferred embodiment the glucoamylase used for the saccharification method of the invention has a residual activity higher that the wild-type A. niger AMG (SEQ ID NO: 2), determined as described in the Materials and Methods section, i.e., determined as residual activity after incubation for 30 minutes in 50 mM NaOAc, pH 4.5, 70.degree. C., 0.2 AGU/ml.

Detail Description Paragraph - DETX (80):
[0135] The glucoamylase enzyme may preferably be derived from a strain of Aspergillus, in particular Aspergillus niger or Aspergillus luchuenesis, a strain of Trichoderma viride, a strain of Rhizopus sp., in particular a strain of Rhizopus niveus, a strain of Endomyces sp., a strain of Cephalosporium cherticola, a strain of Clostridium, in particular Clostridium thermoamylolyticum, Clostridium thermosulphurogenes and Clostridium thermohydrosulphuricum, a strain of Pestalotiopsis, or a strain of Talaromyces, in particular Talaromyces duponti, Talaromyces emersonii and Talaromyces thermophilus.

Detail Description Paragraph - DETX (83):
[0138] Thermostable De-branching Enzymes

Detail Description Paragraph - DETX (84):
[0139] Preferably, the saccharification step of the invention is performed in presence of a thermostable de-branching enzyme. Preferably, the de-branching enzyme is a pullulanase (EC 3.2.1.41) or an isoamylase (EC 3.2.1.68).

Detail Description Paragraph - DETX (85):
[0140] A thermostable pullulanase may be derived from a strain of Bacillus, in particular Bacillus naganoenis or Bacillus acidopullulyticus, a strain of Clostridium, in particular Clostridium thermosulphurogenes and Clostridium thermohydrosulphuricum, or a strain of Pyrococcus, in particular Pyrococcus woesie and Pyrococcus furiosus.

Detail Description Paragraph - DETX (86):
[0141] A thermostable isoamylase may be derived from a strain of Flavobacterium, in particular Flavobacterium odoratum, a strain derived from the thermophilic acrhaebacterium Sulfolobus acidocaldarius (Hayashibara, (1996) Biochimica et Biophysica Acta 1291, p. 177-181, such as Sulfolobus acidocaldarius ATCC33909 and from a strain of Rhodethermus marius.

Detail Description Paragraph - DETX (88):
[0143] In an embodiment, the saccharification step of the invention is performed in presence of a thermostable .alpha.-amylase, preferably a fungal .alpha.-amylase.

Detail Description Paragraph - DETX (92):
[0147] When using a bacterial .alpha.-amylase in the saccharification step of the invention it may suitable be performed in presence of a thermostable bacterial .alpha.-amylase, such as a Bacillus .alpha.-amylase, such as the commercially available B. licheniformis(e.g., Termamyl.TM. from Novo Nordisk) or variants thereof. Another suitable thermostable amylase is the maltogenic amylase from Bacillus stearothermophilus(e.g., Maltogenase.TM. from Novo Nordisk).

Detail Description Paragraph - DETX (99):
[0154] Thermostable CGTases

Detail Description Paragraph - DETX (100):
[0155] When producing cyclodextrins the cyclization step may preferably be performed in the presence of a thermostable CGTase. Suitable thermostable CGTases include the CGTases from the thermophilic anaerobic genus Thermoanaerobacter, the genus Bacillus, such as B. macerans, B. circulars, B. sterothermophilus, and B. subtilis.

Detail Description Paragraph - DETX (170):
[0221] Construction of a thermostable AMG G2 S119P variant Site-directed mutagenesis

Detail Description Paragraph - DETX (184):
[0234] Saccharification using a thermostable AMG variant to produce dextrose liquor.

Claims Text - CLTX (16):
16. The method according to claim 1, in which the saccharification step is performed in presence of a thermostable glucoamylase enzyme (EC 3.2.1.3).

Claims Text - CLTX (22):
22. The method according to claim 16, in which the glucoamylase enzyme derived from a strain of Talaromyces, preferably a strain of Talaromyces emersonii.

Claims Text - CLTX (25):
25. The method according to claim 24, in which the de-branching enzyme is a thermostable pullulanase or a thermostable isoamylase.

Claims Text - CLTX (32):
32. The method according to claim 31, in which the saccharification step is performed in presence of a thermostable MTSase and MTHase.

Claims Text - CLTX (34):
34. The method according to claim 31, in which the liquefied starch is subjected to a thermostable CGTase.

Claims Text - CLTX (36):
36. The method according to claim 31, in which the liquefied starch is subjected to a thermostable .alpha.-amylase and/or pullulanase and/or fungal .alpha.-amylase.

Claims Text - CLTX (62):
62. The method according to claim 46, in which the saccharification step is performed in presence of a thermostable glucoamylase enzyme (EC 3.2.1.3).

Claims Text - CLTX (68):
68. The method according to claim 62, in which the glucoamylase enzyme derived from a strain of Talaromyces, preferably a strain of Talaromyces emersonii.

Claims Text - CLTX (71):
71. The method according to claim 69, in which the de-branching enzyme is a thermostable pullulanase or a thermostable isoamylase.

Claims Text - CLTX (78):
78. The method according to claim 77, in which the saccharification step is performed in presence of a thermostable MTSase and MTHase.

Claims Text - CLTX (80):
80. The method according to claim 77, in which the liquefied starch is subjected to a thermostable CGTase.

Claims Text - CLTX (82):
82. The method according to claim 77, in which the liquefied starch is subjected to a thermostable bacterial .alpha.-amylase and/or pullulanase and/or fungal .alpha.-amylase.

PGPUB-DOCUMENT-NUMBER: 20020155574

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020155574 A1

TITLE: Alpha-amylase mutants with altered properties

PUBLICATION-DATE: October 24, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Thisted, Thomas	Rungsted Kyst		DK	
Kjaerulff, Soren	Vanlose		DK	
Andersen, Carsten	Vaerloese		DK	
Fuglsang, Claus Crone	Niva		DK	

APPL-NO: 09/ 918543

DATE FILED: July 31, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60225140 20000814 US

non-provisional-of-provisional 60233986 20000920 US

non-provisional-of-provisional 60249104 20001116 US

non-provisional-of-provisional 60286869 20010426 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	PA 2000 01160	2000DK-PA 2000 01160	August 1, 2000
DK	PA 2000 01354	2000DK-PA 2000 01354	September 12, 2000
DK	PA 2000 01687	2000DK-PA 2000 01687	November 10, 2000
DK	PA 2001 00655	2001DK-PA 2001 00655	April 26, 2001

US-CL-CURRENT: 435/202, 435/203 , 435/320.1 , 435/325 , 435/69.1

ABSTRACT:

The present invention relates to variants (mutants) of parent Termamyl-like alpha-amylases, which variant has alpha-amylase activity and exhibits altered stability, in particular at high temperatures and/or at low pH relative, and/or low Ca2+ to the parent alpha-amylase.

---------- KWIC ---------

Detail Description Paragraph - DETX (118):

[0149] In another embodient the composition comprises beside a variant of the invention a glucoamylase, in particular a glucoamylase originating from Aspergillus niger (e.g., the G1 or G2 A. niger AMG disclosed in Boel et al. (1984), "Glucoamylases G1 and G2 from Aspergillus niger are synthesized from two different but closely related mRNAs", EMBO J. 3 (5), p. 1097-1102, or a variant therefore, in particular a variant disclosed in WO 00/04136 or WO 01/04273 or the Talaromyces emersonii AMG disclosed in WO 99/28448.

PGPUB-DOCUMENT-NUMBER: 20020006647

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020006647 A1

TITLE: Fermentation with a phytase

PUBLICATION-DATE: January 17, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Veit, Chris	Wake Forest	NC	US	
Felby, Claus	Herlev	NC	DK	
Peckous, Larry W.	Raleigh		US	
Olsen, Hans Sejr	Holte		DK	

APPL-NO: 09/ 788906

DATE FILED: February 20, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60185716 20000223 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	PA 2000 00281	2000DK-PA 2000 00281	February 23, 2000

US-CL-CURRENT: 435/162

ABSTRACT:

The present invention relates to an improved fermenation process wherein phytic acid-containing material is fermented in the presence of a phytase.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. 119 of Danish application PA 2000 00281 filed Feb. 23, 2000 and U.S. Provisional application Ser. No. 60/185,716, filed Feb. 23, 2000, the contents of which are fully incorporated herein by reference.

---------- KWIC ---------

Detail Description Paragraph - DETX (72):
[0078] Other glucoamylases include Talaromyces glucoamylases, preferably derived from Talaromyces emersonii (WO 99/28448), Talaromyces leycettanus (U.S. Pat. No. Re. 32,153), Talaromyces duponti, Talaromyces thermophilus (U.S. Pat. No. 4,587,215). Bacterial glucoamylases preferably include glucoamylases from the genus Clostridium, more preferably, C. thermoamylolyticum (EP 135,138), and C. thermohydrosulfuricum (WO 86/01831).

Detail Description Paragraph - DETX (85):
[0091] This aspect of the invention relates to a composition comprising a phytase and at least one carbohydrate-source generating enzyme (as defined above), preferably, a glucoamylase, such as an Aspergillus niger and/or

Talaromuces emersonii glucoamylase. The composition may further comprise a protease, preferably, an acid protease, such as an acid fungal protease.

US-PAT-NO: 6620924

DOCUMENT-IDENTIFIER: US 6620924 B2

TITLE: Thermostable glucoamylase

DATE-ISSUED: September 16, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Nielsen; Bjarne R.o	Virum	N/A	N/A	DK
slashed.nfeldt	Farum	N/A	N/A	DK
Nielsen; Ruby Ilum	Veks.o slashed.	N/A	N/A	DK
Lehmbeck; Jan				

APPL-NO: 09/ 821616

DATE FILED: March 29, 2001

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of application Ser. No. 09/199,290 filed Nov. 24, 1998, now U.S. Pat. No. 6,255,084 which is a continuation-in-part of application Ser. Nos. 08/979,673 now abandoned and 09/107,657 now abandoned filed Nov. 26, 1997 and Jun. 30, 1998, respectively, and claims priority under 35 U.S.C. 119 of Danish application Nos. 1557/97 and PA 1998 00925 filed Dec. 30, 1997 and Jul. 10, 1998, respectively, and U.S. application Ser. Nos. 60/070,746 and 60/094,344 filed Jan. 8, 1998 and Jul. 28, 1998, respectively, the contents of which are fully incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	1557/97	December 30, 1997
DK	1998 00925	July 10, 1998

US-CL-CURRENT: 536/23.2, 435/205 , 435/252.3 , 435/254.11 , 435/254.3 , 435/320.1

ABSTRACT:

The invention relates to an isolated thermostable glucoamylase derived from Talaromyces emersonii suitable for starch conversion processes.

21 Claims, 15 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

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Abstract Text - ABTX (1):
The invention relates to an isolated thermostable glucoamylase derived from

Talaromyces emersonii suitable for starch conversion processes.

TITLE - TI (1):
Thermostable glucoamylase

Brief Summary Text - BSTX (2):
The present invention relates to a thermostable glucoamylase suitable for, e.g., starch conversion, e.g., for producing glucose from starch. The present invention also relates to the use of said thermostable glucoamylase in various processes, in particular in the saccharification step in starch convention processes.

Brief Summary Text - BSTX (8):
U.S. Pat. No. 4,247,637 describes a thermostable glucoamylase having a molecular weight of about 31,000 Da derived from Talaromyces duponti suitable for saccharifying a liquefied starch solution to a syrup. The glucoamylase is stated to retain at least about 90% of its initial glucoamylase activity when held at 70.degree. C. for 10 minutes at pH 4.5.

Brief Summary Text - BSTX (9):
U.S. Pat. No. 4,587,215 discloses a thermostable amyloglucosidase derived from the species Talaromyces thermophilus with a molecular weight of about 45,000 Da. The disclosed amyloglucosidase (or glucoamylase) loses its enzymatic activity in two distinct phases, an initial period of rapid decay followed by a period of slow decay. At 70.degree. C. (pH=5.0) the half-life for the fast decay is about 18 minutes with no measurable loss of activity within an hour in the second phase of decay.

Brief Summary Text - BSTX (12):
The present invention is based upon the finding of a novel thermostable glucoamylase suitable for use, e.g., in the saccharification step in starch conversion processes.

Brief Summary Text - BSTX (15):
The inventors of the present invention have isolated, purified and characterized a thermostable glucoamylase from a strain of Talaromyces emersonii now deposited with the Centraalbureau voor Schimmelcultures under the number CBS 793.97.

Brief Summary Text - BSTX (20):
The isolated glucoamylase has a very high thermal stability in comparison to prior art glucoamylases, such as the Aspergillus niger glucoamylase (available from Novo Nordisk A/S under the trade name AMG). The T1/2 (half-life) was determined to be about 120 minutes at 70.degree. C. (pH 4.5) as described in Example 2 below. The T1/2 of the recombinant T. emersonii AMG expressed in yeast was determined to be about 110 minutes as described in Example 12.

Brief Summary Text - BSTX (29):
Finally, the invention relates to an isolated pure culture of the microorganism Talaromyces emersonii CBS 793.97 or a mutant thereof capable of producing a glucoamylase of the invention.

Drawing Description Text - DRTX (2):
FIG. 1 shows the SDS-PAGE gel (stained with Coomassie Blue) used for determining the molecular weight (M.sub.w) of the purified Talaromyces emersonii CBS 793.97 glucoamylase of the present invention. 1: Standard marker, 2: Q Sepharose pool (1. run) 3: S Sepharose pool;

Drawing Description Text - DRTX (3):

FIG. 2 shows the pH activity profile of Talaromyces emersonii and Aspergillus niger glucoamylase (AMG) in 0.5% maltose at 60.degree. C.;

Drawing Description Text - DRTX (4):
FIG. 3 shows the temperature activity profile of the Talaromyces emersonii CBS 793.97 glucoamylase vs. Aspergillus niger glucoamylase (AMG)

Drawing Description Text - DRTX (5):
FIG. 4 shows the curve for determining T.sub.1/2 (half-life) in 50 mM NaOAc, 0.2 AGU/ml, pH 4.5, at 70.degree. C. of Talaromyces emersonii CBS 793.97 glucoamylase vs. Aspergillus niger glucoamylase (AMG);

Drawing Description Text - DRTX (12):
FIG. 10 shows the SDS page gel of two transformants, JaL228#5.77 and HowB112#8.10, expressing the Talaromyces emersonii glucoamylase of the invention. JaL228 and HowB112 are the untransformed parent strains. MW: Promega's Protein Molecular;

Drawing Description Text - DRTX (15):
FIG. 13 shows the result of the test for determining the thermostability of recombinant Talaromyces emersonii AMG produced in yeast at 70.degree. C., pH 4.5, 0.2 AGU/ml. T.sub.1/2 was determined to about 110.degree. C.

Detailed Description Text - DETX (2):
The present invention is based upon the finding of a novel thermostable glucoamylase suitable for use in, e.g., the saccharification step in a starch conversion process.

Detailed Description Text - DETX (3):
The inventors of the present invention have isolated, purified and characterized a glucoamylase from a strain of Talaromyces emersonii CBS 793.97. The glucoamylase turned out to have a very high thermal stability in comparison to prior art glucoamylases.

Detailed Description Text - DETX (5):
T.sub.1/2 (half-life) of the isolated Talaromyces emersonii CBS 793.97 glucoamylase was determined to be about 120 minutes at 70.degree. C. as described in Example 2 below and to be about 110.degree. C. for the T. emersonii produced in yeast as described in Example 12.

Detailed Description Text - DETX (12):
Talaromyces emersonii Glucoamylase Amino Acid Sequence

Detailed Description Text - DETX (13):
The inventors have sequenced the thermostable glucoamylase derived from Talaromyces emersonii CBS 793.97 as will be described further in the Example 3 below. According to the invention the Talaromyces AMG may have a Asp145Asn (or D145N) substitution (using SEQ ID NO: 7 numbering).

Detailed Description Text - DETX (28):
The present invention provides a method of using the thermostable glucoamylase of the invention for producing glucose and the like from starch. Generally, the method includes the steps of partially hydrolyzing precursor starch in the presence of .alpha.-amylase and then further hydrolyzing the release of D-glucose from the non-reducing ends of the starch or related oligo- and polysaccharide molecules in the presence of glucoamylase by cleaving .alpha.-(1.degree.4) and .alpha.-(1.degree.6) glucosidic bonds.

Detailed Description Text - DETX (31):

By using a thermostable glucoamylase of the invention saccharification processes may be carried out at a higher temperature than traditional batch saccharification processes. According to the invention saccharification may be carried out at temperatures in the range from above 60-80.degree. C., preferably 63-75.degree. C. This applies both for traditional batch processes (described above) and for continuous saccharification processes.

Detailed Description Text - DETX (32):
Actually, continuous saccharification processes including one or more membrane separation steps, i.e., filtration steps, must be carried out at temperatures of above 60.degree. C. to be able to maintain a reasonably high flux over the membrane. Therefore, a thermostable glucoamylase of the invention provides the possibility of carrying out large scale continuous saccharification processes at a fair price within and period of time acceptable for industrial saccharification processes. According to the invention the saccharification time may even be shortened.

Detailed Description Text - DETX (33):
The activity of a glucoamylase of the invention is generally substantially higher at temperatures between 60.degree. C.-80.degree. C. than at the traditionally used temperature between 30-60.degree. C. Therefore, by increasing the temperature at which the glucoamylase operates the saccharification process may be carried out within a shorter period of time or the process may be carried out using lower enzyme dosage.

Detailed Description Text - DETX (35):
By using a glucoamylase with increased specific activity (measured as activity towards maltose), a lower enzyme dosage may be required in the saccharification process.

Detailed Description Text - DETX (46):
Glucoamylase derived from the deposited filamentous fungus Talaromyces emersonii CBS No. 793.97 has been deposited with the Centraalbureau voor Schimmelcultures, P.O. Box 273, 3740 AG Baarn, the Netherlands, for the purposes of patent procedure on the date indicated below. CBS being an international depository under the Budapest Treaty affords permanence of the deposit in accordance with rule 9 of said treaty. Deposit date: Jun. 2, 1997 Depositor's ref.: NN049253 CBS designation: CBS 793.97

Detailed Description Text - DETX (54):
T. emersonii glucoamylase gene with introns is shown in FIG. 5 and SEQ ID NO: 33. The introns are shown in FIG. 5.

Detailed Description Text - DETX (103):
Characterisation of the Talaromyces emersonii Glucoamylase

Detailed Description Text - DETX (104):
The purified Talaromyces emersonii CBS 793.97 glucoamylase was used for characterisation.

Detailed Description Text - DETX (110):
The pH-activity dependency of the Talaromyces emersonii glucoamylase was determined and compared with profile of Aspergillus niger glucoamylase.

Detailed Description Text - DETX (113):
The temperature-activity dependency of the Talaromyces emersonii glucoamylase of the invention was determined and compared with the profile of Aspergillus niger glucoamylase.

Detailed Description Text - DETX (116):
The thermal stability of the Talaromyces emersonii glucoamylase was determined and compared with the thermal stability of Aspergillus niger glucoamylase.

Detailed Description Text - DETX (118):
The T1/2 of the Talaromyces emersonii glucoamylase was determined to about 120 minutes at 70.degree. C. The T1/2 of the Aspergillus niger glucoamylase was determined to 7 minutes under the same conditions (See FIG. 4).

Detailed Description Text - DETX (122):
Sequencing of the N-terminal of T. emersonii Glucoamylase

Detailed Description Text - DETX (123):
The N-terminal amino acid sequence of T. emersonii glucoamylase was determined following SDS-PAGE and electroblotting onto a PVDF-membrane. Peptides were derived from reduced and S-carboxymethylated glucoamylase by cleaving with a lysyl-specific protease. The resulting peptides were fractionated and re-purified using RP-HPLC before subjected to N-terminal sequence determination.

Detailed Description Text - DETX (125):
The Full Length T. emersonii Glucoamylase

Detailed Description Text - DETX (126):
The full length T. emersonii glucoamylase amino acid sequence shown in SEQ ID NO: 7 was identified using standard methods.

Detailed Description Text - DETX (128):
Cloning and Sequencing of the Talaromyces emersonii Glucoamylase Gene

Detailed Description Text - DETX (178):
Expression of Talaromyces emersonii Glucoamylase in Yeast

Detailed Description Text - DETX (181):
The yeast cells were grown at 30.degree. C. for 3 days in Sc-ura medium followed by growth for 3 days in YPD. The culture was then centrifuged and the supernatant was used for the thermostability assay described in the "Materials and Method" section.

Detailed Description Text - DETX (186):
200 ml culture broth from fermentation of A. niger HowB112 harboring the Talaromyces emersonii gene was centrifuged at 9000 rpm and dialyzed against 20 mM NaOac, pH 5 over night. The solution was then applied on a S Sepharose column (200 ml) previously equilibrated in 20 mM NaOAc, pH 5. The glucoamylase was collected in the effluent, and applied on a Q Sepharose column (50 ml) previously equilibrated in 20 mM NaOAC, pH 4.5. Unbound material was washed of the column and the glucoamylase was eluted using a linear gradient from 0-0.3 M NaCl in 20 mM NaOAc over 10 column volumes. Purity of the glucoamylase fraction was checked by SDS-PAGE and only one single band was seen. The molecular weight was again found to about 70 kdal as seen for the wild type glucoamylase. The specific activity towards maltose was measured and a specific activity of 8.0 AGU/mg (37.degree. C.) and 21.0 AGU/mg (60.degree. C.) were found which is in accordance the data on the wild type enzyme.

Detailed Description Text - DETX (192):
The saccharification performance of the Talaromyces emersonii glucoamylase was tested at different temperatures with and without the addition of acid .alpha.-amylase and pullulanase. Saccharification was run under the following

conditions:

Detailed Description Text - DETX (199):
The thermal stability of recombinant Talaromyces emersonii glucoamylase expressed in yeast (purified using the method described in Example 9) was determined at 70.degree. C., pH 4.5, 0.2 AGU/ml using the method described above in the "Material and Methods" section as "Thermal Stability I (T1/2 (half-life) determination of AMG".

Detailed Description Text - DETX (200):
FIG. 13 shows the result of the test. The T1/2 of the recombinant Talaromyces emersonii glucoamylase expressed in yeast was determined to about 110 minutes at 70.degree. C.

Detailed Description Paragraph Table - DETL (12):
Substrate: 10 DE Maltodextrin, approx. 30% DS (w/w) Temperatures: 60, 65, or 70.degree. C. Initial pH: 4.5 Enzyme Dosage: Recombinant Talaromyces emersonii glucoamylase produced in A. niger: 0.24 or 0.32 AGU/g DS Acid .alpha.-amylase derived from A. niger: 0.020 AFAU/g DS Pullulanase derived from Bacillus: 0.03 PUN/g DS When used alone Talaromyces AMG was dosed at the high dosage (0.32 AGU/g DS), otherwise at the low dosage, i.e., 0.24 AGU/g DS.

Claims Text - CLTX (1):
1. An isolated DNA sequence encoding an enzyme with glucoamylase activity, wherein the enzyme [(a) is derived from Talaromyces and has a T1/2 (half-life) of at least 100 minutes in 50 mM NaOAc, 0.2 Novo Amyloglucosidase Unit (AGU)/ml, pH 4.5, at 70.degree. C. or (b)] has an amino acid sequence that has at least 80% identity with the glucoamylase of SEQ ID NO:7.

US-PAT-NO: 6573086

DOCUMENT-IDENTIFIER: US 6573086 B1

TITLE: Transformation system in the field of filamentous fungal hosts

DATE-ISSUED: June 3, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Emalfrab; Mark Aaron	Jupiter	FL	N/A	N/A
Burlingame; Richard Paul	Manitowoc	WI	N/A	N/A
Olson; Philip Terry	Manitowoc	WI	N/A	N/A
Sinitsyn; Arkady	Moscow	N/A	N/A	RU
Panteleimonovich	Toulouse	N/A	N/A	FR
Parriche; Martine	Quint-Fonsegrives	N/A	N/A	FR
Bousson; Jean Christophe	Manitowoc	WI	N/A	N/A
Pynnonen; Christine Marie	Houten	N/A	N/A	NL
Punt; Peter Jan	Vieuten-De Meern	N/A	N/A	NL
Van Zeijl; Cornelia Marie Johanna				

APPL-NO: 09/ 548938

DATE FILED: April 13, 2000

PARENT-CASE:

REFERENCE TO PRIOR APPLICATIONS

This is a continuation-in-part of international application PCT/NL99/00618, filed Oct. 6, 1999, which is a continuation-in-part of international application PCT/EP98/06496, filed Oct. 6, 1998.

US-CL-CURRENT: 435/254.11, 435/209 , 435/69.1

ABSTRACT:

A novel transformation system in the field of filamentous fungal hosts for expressing and secreting heterologous proteins or polypeptides is described. The invention also covers a process for producing large amounts of polypeptide or protein in an economical manner. The system comprises a transformed or transfected fungal strain of the genus Chrysosporium, more particularly of Chrysosporium lucknowense and mutants or derivatives thereof. It also covers transformants containing Chrysosporium coding sequences, as well expression-regulating sequences of Chrysosporium genes. Also provided are novel fungal enzymes and their encoding sequences and expression-regulating sequences.

25 Claims, 69 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 36

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Brief Summary Text - BSTX (42):

An expression-regulatingregion is a DNA sequence recognised by the host Chrysosporium strain for expression. It comprises a promoter sequence operably linked to a nucleic acid sequence encoding the polypeptide to be expressed. The promoter is linked such that the positioning vis-a-vis the initiation codon of the sequence to be expressed allows expression. The promoter sequence can be constitutive or inducible. Any expression regulating sequence or combination thereof capable of permitting expression of a polypeptide from a Chrysosporium strain is envisaged. The expression regulating sequence is suitably a fungal expression-regulatingregion e.g. an ascomycete regulating region. Suitably the fungal expression regulating region is a regulating region from any of the following genera of fungi: Aspergillus, Trichoderma, Chrysosporium (preferred), Hansenula, Mucor, Pichia, Neurospora, Tolypocladium, Rhizomucor, Fusarium, Penicillium, Saccharomyces, Talaromyces or alternative sexual forms thereof like Emericella, Hypocrea e.g. the cellobiohydrolase promoter from Trichoderma, glucoamylase promoter from Aspergillus, glyceraldehyde phosphate dehydrogenase promoter from Aspergillus, alcohol dehydrogenase A and alcohol dehydrogenase R promoter of Aspergillus, TAKA amylase promoter from Aspergillus, phosphoglycerate and cross-pathway control promoters of Neurospora, aspartic proteinase promoter of Rhizomucor miehei, lipase promoter of Rhizomucor miehei and beta-galactosidase promoter of Penicillium canescens. An expression regulating sequence from the same genus as the host strain is extremely suitable, as it is most likely to be specifically adapted to the specific host. Thus preferably the expression regulating sequence is one from a Chrysosporium strain.

Brief Summary Text - BSTX (53):

Suitable examples of signal sequences can be derived from yeasts in general or any of the following specific genera of fungi: Aspergillus, Trichoderma, Chrysosporium, Pichia, Neurospora Rhizomucor, Hansenula, Humicola, Mucor, Tolypocladium, Fusarium, Penicillium, Saccharomyces, Talaromyces or alternative sexual forms thereof like Emericella, Hypocrea. Signal sequences that are particularly useful are often natively associated with the following proteins a cellobiohydrolase, an endoglucanase, a beta-galactosidase,a xylanase, a pectinase, an esterase, a hydrophobin, a protease or an amylase. Examples include amylase or glucoamylase of Aspergillus or Humicola (4), TAKA amylase of Aspergillus oryzae, alpha-amylase of Aspergillus niger, carboxyl peptidase of Mucor (U.S. Pat. No. 5,578,463), a lipase or proteinase from Rhizomucor miehei, cellobiohydrolase of Trichoderma (5), beta-galactosidase of Penicillium canescens and alpha mating factor of Saccharomyces.

US-PAT-NO: 6448049

DOCUMENT-IDENTIFIER: US 6448049 B1

TITLE: Starch conversion process

DATE-ISSUED: September 10, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tsutsumi; Noriko	Chiba-ken	N/A	N/A	JP
Bisg.ang.rd-Frantzen; Henrik	Bagsv.ae butted.rd	N/A	N/A	DK
Svendsen; Allan	Birker.o slashed.d	N/A	N/A	DK

APPL-NO: 09/ 544123

DATE FILED: April 6, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a divisional of application Ser. No. 09/129,075 filed on Aug. 4, 1998, now U.S. Pat. No. 6,087,149 which is a continuation of PCT/DK98/00304 filed Jul. 2, 1998, and claims priority under 35 U.S.C. 119 of Danish application no. 0787/97 filed on Jul. 2, 1997, and U.S. application Ser. No. 60/055,567 filed Aug. 13, 1997, the contents of which are fully incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	0787/97	February 7, 1997

US-CL-CURRENT: 435/98, 435/210

ABSTRACT:

The present invention relates to a starch conversion process of the type which includes a debranching step wherein an isoamylase being active at the process conditions prevailing is used for debranching the starch and to the use of thermostable isoamylases for starch conversion. The invention further relates to an isolated isoamylase obtained from a strain of the genus Rhodothermus and to cloned DNA sequences encoding isoamylases derived from a strain of Rhodothermus or Sulfolobus, to expression vectors comprising said DNA sequence, host cells comprising such expression vectors, and finally to methods for producing said isoamylases.

4 Claims, 7 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 7

---------- KWIC ---------

Abstract Text - ABTX (1):

The present invention relates to a starch conversion process of the type which includes a debranching step wherein an isoamylase being active at the process conditions prevailing is used for debranching the starch and to the use of thermostable isoamylases for starch conversion. The invention further relates to an isolated isoamylase obtained from a strain of the genus Rhodothermus and to cloned DNA sequences encoding isoamylases derived from a strain of Rhodothermus or Sulfolobus, to expression vectors comprising said DNA sequence, host cells comprising such expression vectors, and finally to methods for producing said isoamylases.

Brief Summary Text - BSTX (2):

The invention relates to a starch conversion process of the type which includes a debranching step. The invention also relates to the use of a thermostable isoamylase for debranching starch. The invention further relates to an isolated isoamylase obtained from a strain of the genus Rhodothermus, to cloned DNA sequences encoding isoamylases derived from a strain of Rhodothermus or Sulfolobus, to expression vectors comprising said DNA sequence, host cells comprising such expression vectors, and finally to methods for producing said isoamylases.

Brief Summary Text - BSTX (32):

It is the object of the present invention to provide a starch conversion process of the type which includes a debranching step which results in a reduced formation of the undesired trisaccharide panose. The invention also relates to a novel thermostable isoamylase suitable for use in the starch conversion process of the invention.

Brief Summary Text - BSTX (36):

In the second aspect the invention relates to the use of a thermostable isoamylase in starch conversion processes.

Detailed Description Text - DETX (2):

It is the object of the present invention to provide a starch conversion process of the type which includes a debranching step which results in a reduced formation of the undesired trisaccharide panose. The invention also relates to a novel thermostable isoamylase suitable for use in the starch conversion process of the invention.

Detailed Description Text - DETX (7):

A thermostable isoamylase makes it possible to perform the liquefaction and the debranching at the same time before the saccharification step.

Detailed Description Text - DETX (8):

Specific examples of thermostable debranching enzymes are the thermostable isoamylases derived from the thermophilic bacteria such as Sulfolobus acidocaldarius ATCC 33909 (Maruta, K. et al., Biochimica et Biophysica Acta 1291, p. 177-181 (1996)), Sulfolobus solfataricus ATCC 35092 (accession number: Y08256) and Rhodothermus marinus DSM 4252 as will be described further below.

Detailed Description Text - DETX (12):

In a preferred embodiment the debranching enzyme being active at the process conditions prevailing is a thermostable isoamylase.

Detailed Description Text - DETX (18):

Isoamylases which can be used according to the invention include the thermostable isoamylase derived from the thermophilic archaebacteria Sulfolobus acidocaldarius, Sulfolobus solfataricus and the thermophilic eubacterium Rhodothermus marinus (as will be described in details below).

Detailed Description Text - DETX (25):
In the second aspect the invention relates to the use of a thermostable isoamylase in starch conversion processes, including fructose syrup conversion processes and for producing fat replacers.

Detailed Description Text - DETX (26):
In the case of the starch conversion process is a starch depolymerization process the thermostable isoamylase is used in combination with an .alpha.-amylase during the liquefaction step. The thermostable isoamylase may be derived from the thermophilic archaebacteria Sulfolobus acidocaldarius or Sulfolobus solfataricus or from Rhodothermus marinus.

Detailed Description Text - DETX (27):
The thermostable isoamylase may be used during the liquefaction step of a starch to glucose syrup or fructose syrup conversion process. The thermostable isoamylase may also be used in processes for producing fat replacers from starch.

Detailed Description Text - DETX (30):
Several advantages are obtained by the addition of a thermostable isoamylase.

Detailed Description Text - DETX (32):
As the debranching enzyme is a thermostable isoamylase the debranching by a pullulanase during saccharification can be left out. This is advantageous as pullulanase tends to condense maltose into a panose precursor, 6.sup.2 -.alpha.-maltosylmaltose which is hydrolysed into panose by glucoamylase.

Detailed Description Text - DETX (33):
By prolonging the liquefaction step the DE is increased from 10-15 to e.g. 15-20 reducing the need for glucoamylase (e.g. AMG.TM.). This reduced glucoamylase requirement is advantageous as the formation of isomaltose is reduced. Isomaltose is formed from D-glucose resulting from depolymerization of linear oligosaccharides by glucoamylase which removes D-glucose from the non-reducing chain-ends. All together less glucoamylase activity results in an increased glucose yield.

Detailed Description Text - DETX (39):
When using a thermostable isoamylase up front in the liquefaction process the process is less dependent of the specificity of the .alpha.-amylase and the formation of the panose precursors. This change allows an increased process time for the liquefaction and a higher DX than the normal value of DX 10-12 is obtained. If a more intensive liquefaction is allowed and a higher DX value is obtained by the use of a thermostable debranching enzyme its possible to increase the concentration of Dry Substance (DS) from the normal 30-35% to a higher percentage. Such an increase in % DS is advantageous as the evaporation costs are reduced significantly downstream in the High Fructose Corn Syrup (HFCS) process.

Detailed Description Text - DETX (40):
How to Identify Suitable Thermostable Isoamylases

Detailed Description Text - DETX (41):
Suitable thermostable isoamylases may be identified as described in Example 1 by first identifying conserved regions of amino acid sequence by aligning isoamylase sequences available. On the basis of the conserved regions PCR primers are designed. Genomic DNA stocks of a number of bacterial strains are then subjected to PCR, and strains yielding a fragment of the expected size are selected. The fragments are purified, sequenced, and aligned to confirm the

homology with published isoamylase sequences. Among the strains identified, ones with the highest optimumgrowth temperature are further selected.

Detailed Description Text - DETX (60):
Characterisation of the Novel Thermostable Isoamylase from Rhodothermus marinus

Detailed Description Text - DETX (163):
To provide thermostable isoamylases an alignment of five known isoamylases of which the protein sequence data is available was made (see Table 1).

Detailed Description Text - DETX (176):
Cloning of a Gene Encoding a Thermostable Isoamylase and its Expression in E.coli

US-PAT-NO: 6352851

DOCUMENT-IDENTIFIER: US 6352851 B1

TITLE: Glucoamylase variants

DATE-ISSUED: March 5, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Nielsen; Bjarne Roenfeldt	Virum	N/A	N/A	DK
Svendsen; Allan	Birkeroed	N/A	N/A	DK
Pedersen; Henrik	Odense	N/A	N/A	DK
Vind; Jesper	Lyngby	N/A	N/A	DK
Hendriksen; Hanne Vang	Holte	N/A	N/A	DK
Frandsen; Torben Peter	Frederiksberg	N/A	N/A	DK

APPL-NO: 09/ 351814

DATE FILED: July 12, 1999

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 U.S.C. 119 of Danish application Nos. PA 1998 00937 and PA 1998 01667 filed Jul. 15, 1998 and Dec. 17, 1998 and U.S. provisional application Nos. 60/093,528 and 60/115,545 filed Jul. 21, 1998 and Jan. 12, 1999, the contents of which are fully incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	1998 00937	July 15, 1998
DK	1998 01667	December 17, 1998

US-CL-CURRENT: 435/205, 435/183 , 435/200 , 435/202 , 435/203

ABSTRACT:

The invention relates to a variant of a parent fungal glucoamylase, which exhibits improved thermal stability and/or increased specific activity using saccharide substrates.

6 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

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Brief Summary Text - BSTX (13):

The object of the present invention is to provide improved glucoamylase variants with improved thermostability and/or increased specific activity suitable for use in, e.g., the saccharification step in starch conversion

processes.

Brief Summary Text - BSTX (14):
The term "a glucoamylase variant with improved thermostability" means in the context of the present invention a glucoamylase variant which has a higher T.sub.1/2 (half-time) than the corresponding parent glucoamylase. The determination of T1/2 (Method I and Method II) is described below in the "Materials & Methods" section.

Brief Summary Text - BSTX (15):
The term "a glucoamylase variant with increased specific activity" means in the context of the present invention a glucoamylase variant with increased specific activity towards the .alpha.-1,4 linkages in the saccharide in question. The specific activity is determined as k.sub.cat or AGU/mg (measured as described below in the "Materials & Methods" section). An increased specific activity means that the k.sub.cat or AGU/mg values are higher when compared to the k.sub.cat or AGU/mg values, respectively, of the corresponding parent glucoamylase.

Brief Summary Text - BSTX (16):
The inventors of the present invention have provided a number of improved variants of a parent glucoamylase with improved thermostability and/or increased specific activity in comparison to the parent corresponding enzyme. The improved thermal stability is obtained by substituting selected positions in a parent glucoamylase. This will be described in details below.

Detailed Description Text - DETX (2):
A goal of the work underlying the present invention was to improve the thermal stability and/or increase the specific activity of particular glucoamylases which are obtainable from fungal organisms, in particular strains of the Aspergillus genus and which themselves had been selected on the basis of their suitable properties in starch conversion or alcohol fermentation.

Detailed Description Text - DETX (3):
Identifying Positions and/or Regions to be Mutated to Obtain Improved Thermostability and/or Increased Specific Activity

Detailed Description Text - DETX (15):
Regions found to be of interest for increasing the specific activity and/or improved thermostability are the regions in 1 proximity to the active site. Regions positioned in between the .alpha.-helixes, and which may include positions on each side of the N-and C-terminal of the .alpha.-helixes, at the substrate binding site is of importance for the activity of the enzyme. These regions constitute the following regions:

Detailed Description Text - DETX (26):
The present inventors have found that it is in fact possible to improve the thermal stability and/or to increase the specific activity of a parent glucoamylase by modification of one or more amino acid residues of the amino acid sequence of the parent glucoamylase. The present invention is based on this finding.

Detailed Description Text - DETX (29):
Parent glucoamylase contemplated according to the present invention include fungal glucoamylases, in particular fungal glucoamylases obtainable from an Aspergillus strain, such as an Aspergillus niger or Aspergillus awamori glucoamylases and variants or mutants thereof, homologous glucoamylases, and further glucoamylases being structurally and/or functionally similar to SEQ ID NO: 2. Specifically contemplated are the Aspergillus niger glucoamylases G1

and G2 disclosed in Boel et al. (1984), "Glucoamylases G1 and G2 from Aspergillus niger are synthesized from two different but closely related mRNAs", EMBO J. 3 (5), p. 1097-1102. The G2 glucoamylase is disclosed in SEQ ID NO: 2. The G1 glucoamylase is disclosed in SEQ ID NO: 13. Another AMG backbone contemplated is Talaromyces erersonii, especially Talaromyces emersonii DSM disclosed in WO 99/28448 (Novo Nordisk).

Detailed Description Text - DETX (273):
As substrate binding may improve the stability region 93-127, Region: 170-184, Region: 305-319 are also contemplated for thermostabilization according to the present invention.

Detailed Description Text - DETX (434):
In a third aspect the invention relates to a variant of a parent glucoamylase with increased specific activity comprising one or more mutation(s) in the following position(s) or region(s) in the amino acid sequence shown in NO: 2:

Detailed Description Text - DETX (602):
By using a thermostable glucoamylase variant of the invention saccharification processes may be carried out at a higher temperature than traditional batch saccharification processes. According to the invention saccharification may be carried out at temperatures in the range from above 60-80.degree. C., preferably 63-75.degree. C. This apply both for traditional batch processes (described above) and for continuous saccharification processes.

Detailed Description Text - DETX (603):
Actually, continuous saccharification processes including one or more membrane separation steps, i.e. filtration steps, must be carried out at temperatures of above 60.degree. C. to be able to maintain a reasonably high flux over the membrane or to minimize microbial contamination. Therefore, the thermostable variants of the invention provides the possibility of carrying out large scale continuous saccharification processes at a fair price and/or at a lower enzyme protein dosage within a period of time acceptable for industrial saccharification processes. According to the invention the saccharification time may even be shortened.

Detailed Description Text - DETX (607):
A glucoamylase with an increased specific activity towards saccharides present in the solution after liquefaction and saccharides formed during saccharification would be an advantage as a reduced enzyme protein dosage or a shorter process time then could be used. In general, the glucoamylase has a preference for substrates consisting of longer saccharides compared to short chain saccharides and the specific activity towards e.g. maltoheptaose is therefore approximately 6 times higher than towards maltose. An increased specific activity towards short chain saccharides such as maltose (without reducing the activity towards oligosaccharides) would therefore also permit using a lower enzyme dosage and/or shorter process time.

Detailed Description Text - DETX (608):
Furthermore, a higher glucose yield can be obtained with a glucoamylase variant with an increased alpha-1,4 hydrolytic activity (if the alpha-1,6 activity is unchanged or even decreased), since a reduced amount of enzyme protein is being used, and alpha-1,6 reversion product formation therefore is decreased (less isomaltose).

Detailed Description Text - DETX (614):
wherein the enzymatic saccharification is carried out using a thermostable

glucoamylase variant of the invention.

Detailed Description Text - DETX (682):
Screening For Thermostable AMG Variants

Detailed Description Text - DETX (683):
The libraries are screened in the thermostable filter assay described below.

Detailed Description Text - DETX (684):
Filter Assay For Thermostability

Detailed Description Text - DETX (721):
Construction, by Localized Random, Doped Mutagenesis, of A. niger AMG Variants Having Improved Thermostability Compared to the Parent Enzyme

Detailed Description Text - DETX (722):
To improve the thermostability of the A. niger AMG random mutagenesis in pre-selected region was performed.

Detailed Description Text - DETX (794):
The library was screened in the thermostability filter assays using a Protran filter and incubating at 67-69.degree. C. as described in the "Material & Methods" section above.

Detailed Description Text - DETX (796):
Construction, by PCR Shuffling Spiked with DNA Oligos, of A. niger AMG Variants Having Improved Thermostability Compared to the Parent Enzyme

Detailed Description Text - DETX (943):
Thermostability at 70.degree. C.

Detailed Description Text - DETX (945):
The thermostability was determined as T.sub.1/2 using Method I, and as % residual activity after incubation for 30 minutes in 50 mM NaOAc, pH 4.5, 70.degree. C., 0.2 AGU/ml, as described in the "Material & Methods" section above. The result of the tests are in the Table below and compared to the wild-type A. niger AMG G2.

Detailed Description Text - DETX (947):
Thermostability at 68.degree. C.

Detailed Description Text - DETX (949):
The thermostability was determined as T1/2 using method I at 68.degree. C. as described in the "Materials & Methods" section and compared to the wild-type A. niger AMG G2 under the same conditions. Evaluation of variants were performed on culture broth after filtration of the supernatants.

Detailed Description Text - DETX (951):
Thermostability at 68.degree. C.

Detailed Description Text - DETX (955):
Thermostability at 68.degree. C.

Detailed Description Text - DETX (956):
AMG G2 variants were constructed using the approach described in Example 3. The thermostability was determined as T1/2 using method I at 68.degree. C. as described in the "Materials & Methods" section and compared to the wild-type A. niger AMG G2 under the same conditions. Evaluation of variants were performed on culture broth after filtration of the supernatants.

Detailed Description Text - DETX (958):
Thermostability at 70.degree. C.

Detailed Description Text - DETX (960):
The thermostability was determined as % residual activity using Method I in 50 mM NaOAc, pH 4.5, 70.degree. C., as described in the "Material & Methods" section above. The result of the test is listed in the Table below and compared to the wild-type A. niger AMG G2.

Detailed Description Text - DETX (962):
Thermostability at 70.degree. C. in Presence of 30% Glucose

Detailed Description Text - DETX (964):
The thermostability was determined as T1/2 using method II at 70.degree. C. as described in the "Materials & Methods" section and compared to the wild-type A. niger AMG G2 under the same conditions.

Detailed Description Text - DETX (967):
Saccharification performance of the AMG variants S119P+Y402F+S411V and PLASD(N-terminal)+V59A+A393R+T490A, respectively, both having improved thermostability are tested at 70.degree. C. as described below.

Detailed Description Paragraph Table - DETL (7):
T1/2 A. niger AMG G2 T1/2 (wild type) Variant (min) (min) 1 A246T + T72I 11.3 8.5 2 G447S + S119P 11.4 7.9 3 E408R + A425T + S465P + T494A 8.6 8.1 4 E408R + S386N 12.6 8.9 5 T2P 9.3 8.5 6 T2Q + A11P + S394R 10.7 8.5 7 T2H 9.5 8.9 8 A11E + E408R 12.7 9.3 9 T2M + N9A + T390R + D406N + L410R 10.7 8.5 10 A393R 17.7 8.4 11 T2R + S386R + A393R 14.1 8.4 12 A393R + L410R 14.7 7.9 13 A1V + L66R + Y402F + N427S + S486G 11.7 8.5 14 T2K + S30P + N427M + S444G + 11.4 8.4 V470M Thermostability at 70.degree. C. on purified samples. T1/2 Enzyme (min) 15 AMG G2 (wild type) 7.4 16 T2E + T379A + S386K + A393R 11.6 17 E408R + S386N 10.2 18 T2Q + A11P + S394R 9.8 19 A1V + L66R + Y402F + N427S + S486G 14.1 20 A393R 14.6 21 T2R + S386R + A393R 14.1 22 A393R + L410R 12.9 23 Y402F 10.1

Other Reference Publication - OREF (17):
Abstract of article: Mutational modulation of substrate bond-type specificity and thermostability of glucoamylase from Aspergillus awamori by replacement with short homologue active site sequences and thiol/disulfide engineering, Biochem., vol. 35, pp. 8696-8704 (1996).

US-PAT-NO: 6329182

DOCUMENT-IDENTIFIER: US 6329182 B1

TITLE: Method of producing oligosaccharide syrups, a system for producing the same and oligosaccharide syrups

DATE-ISSUED: December 11, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Pedersen; Sven	Gentofte	N/A	N/A	DK
Hendriksen; Hanne Vang	Holte	N/A	N/A	DK

APPL-NO: 09/ 200109

DATE FILED: November 25, 1998

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	1356/97	November 26, 1997

US-CL-CURRENT: 435/96, 127/55 , 210/651 , 210/652 , 210/654 , 435/100 , 435/95 , 435/99

ABSTRACT:

The present invention relates to a method of producing oligosaccharide syrups, in particular to the production of syrups having a high concentration of saccharides with a degree of polymerization of at least 2, comprising the steps of: enzymatic reaction of a substrate at a temperature in the range of 50.degree. C. to 100.degree. C. obtaining a saccharide solution comprising monosaccharides and disaccharides, trisaccharides and higher saccharides; nanofiltration of the saccharide solution at a temperature in the range of 60.degree. C. to 100.degree. C. obtaining a syrup essentially comprising disaccharides, trisaccharides and higher saccharides; recovering said syrup; optionally recycling the permeate resulting from the nanofiltration step to the enzymatic reaction.

20 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

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Detailed Description Text - DETX (21):

In a preferred embodiment of the present invention heatstable glucoamylase enzymes are utilised for the enzymatic reaction. The glucoamylase enzyme may preferably be derived from a strain of Aspergillus, in particular Aspergillus niger, a strain of Clostridium, in particular Clostridium thermoamylolyticum, Clostridium thermosulphurogenes, Clostridium thermohydrosulphuricum, a strain of Pestalotiopsis, or a strain of Talaromyces, in particular Talaromyces duponti, Talaromyces emersonii and Talaromyces thermophilus.

US-PAT-NO: 6309872

DOCUMENT-IDENTIFIER: US 6309872 B1

TITLE: Polypeptides having glucoamylase activity and nucleic acids encoding same

DATE-ISSUED: October 30, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Rey; Michael W.	Davis	CA	N/A	N/A
Golightly; Elizabeth J.	Davis	CA	N/A	N/A

APPL-NO: 09/ 704449

DATE FILED: November 1, 2000

US-CL-CURRENT: 435/205, 536/23.2

ABSTRACT:

The present invention relates to isolated polypeptides having glucoamylase activity and isolated nucleic acid sequences encoding the polypeptides. The invention also relates to nucleic acid constructs, vectors, and host cells comprising the nucleic acid sequences as well as methods for producing and using the polypeptides.

12 Claims, 17 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 17

---------- KWIC ---------

Brief Summary Text - BSTX (7):
There is a need in the art for new sources of glucoamylase with improved properties of thermostability.

Drawing Description Text - DRTX (6):
FIG. 5 shows the thermostability of a Thielavia terrestris ATCC 20627 glucoamylase.

Detailed Description Text - DETX (41):
Modification of a nucleic acid sequence encoding a polypeptide of the present invention may be necessary for the synthesis of polypeptides substantially similar to the polypeptide. The term "substantially similar" to the polypeptide refers to non-naturally occurring forms of the polypeptide. These polypeptides may differ in some engineered way from the polypeptide isolated from its native source, e.g., variants that differ in specific activity, thermostability, pH optimum, or the like. The variant sequence may be constructed on the basis of the nucleic acid sequence presented as the polypeptide encoding part of SEQ ID NO:1, e.g., a subsequence thereof, and/or by introduction of nucleotide substitutions which do not give rise to another amino acid sequence of the polypeptide encoded by the nucleic acid sequence,

but which correspond to the codon usage of the host organism intended for production of the enzyme, or by introduction of nucleotide substitutions which may give rise to a different amino acid sequence. For a general description of nucleotide substitution, see, e.g., Ford et al., 1991, Protein Expression and Purification 2: 95-107.

Detailed Description Text - DETX (147):
It will be understood that the term "eukaryotic polypeptides" includes not only native polypeptides, but also those polypeptides, e.g., enzymes, which have been modified by amino acid substitutions, deletions or additions, or other such modifications to enhance activity, thermostability, pH tolerance and the like.

Detailed Description Text - DETX (150):
In a still further aspect, the present invention relates to compositions comprising a polypeptide of the present invention. Preferably, the compositions are enriched in a polypeptide of the present invention. In the present context, the term "enriched" indicates that the glucoamylase activity of the composition has been increased, e.g., with an enrichment factor of 1.1.

Detailed Description Text - DETX (216):
The results for the highest producers are shown in Table 1. The highest glucoamylase activity of the transformants was 5 and 3 times that of the untransformed host at 6 and 8 days, respectively.

Detailed Description Text - DETX (239):
Thermostability

Detailed Description Text - DETX (240):
The thermostability of the Thielavia terrestris glucoamylase was determined by incubating a solution 10 .mu.l of the glucoamylase (0.11 mg/ml) and 5 .mu.l of Britton and Robinson pH 4 buffer (in quadruplicate) at 4, 22, 30, 37, 50, 60, 70, and 80.degree. C., respectively, for 30 minutes. The solutions were then chilled (in ice-water) and briefly centrifuged (in 0.7-ml micro-centrifuge tubes) before being transferred to a 96-well microplate for glucoamylase activity assay. One assay was made by adding simultaneously 10 .mu.l of 10 mM maltotriose, 50 .mu.l of glucose oxidase assay reagent, and 25 .mu.l of H.sub.2 0 to the Thielavia terrestris glucoamylase solution to start the reaction, whereas another assay was made by pre-incubating the Thielavia terrestris glucoamylase solution with the maltotriose stock for 3 minutes before adding the glucose oxidase assay reagent and H.sub.2 O.

Detailed Description Text - DETX (242):
The Thielavia terrestris glucoamylase retained 60% of its activity after being incubated at pH 4 and 70.degree. C. for 30 minutes. This thermostability was comparable to that of Aspergillus terreus glucoamylase (Ghose et al., 1990, FEMS Microbiol. Lett. 66: 345-350) but higher than the glucoamylase from Aspergillus hennebergi (Alazard and Baldensperger, 1982, Carbohydrate Res. 107: 231-241), Aspergillus niger (Ono et al., 1988, Agric. Biol. Chem. 52: 1699-1706), Aspergillus spp K-27 (Abe et al., 1990, Carbohydrate Res. 203: 129-138), Aspergillus awamori (Libby et al., 1994, Prot. Engineer. 7: 1109-1114), H. resinae (Fagerstrom et al., 1990, J. Gen. Microbiol. 136: 913-920), T. reesei (Fagerstrom et al., 1995, Biotechnol. Appl. Biochem. 21: 223-231), H. grisea (Campos et al., 1995, Appl. Environ. Microbiol. 61: 2436-2438), and C. paradoxa (Monma et al., 1987, Carbohydrate Res. 159: 255-261).

Detailed Description Text - DETX (246):
The Thielavia terrestris glucoamylase at 11 .mu.g/ml or 0.18 .mu.M showed a

K.sub.m of 0.33.+-.0.07 mM and a k.sub.cat of (5.5.+-.0.5).times.10.sup.3 min.sup.-1 for maltotriose hydrolysis at pH 4. At 37.degree. C. and pH 4.3, the Thielavia terrestris glucoamylase showed a specific activity almost 2-fold higher than that of the Aspergillus niger glucoamylase in hydrolyzing maltose.

US-PAT-NO: 6303346

DOCUMENT-IDENTIFIER: US 6303346 B1

TITLE: Method of producing saccharide preparations

DATE-ISSUED: October 16, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Liaw; Gin C.	Decatur	IL	N/A	N/A
Pedersen; Sven	Gentofte	N/A	N/A	DK
Hendriksen; Hanne Vang	Holte	N/A	N/A	DK
Svendsen; Allan	Birker.o slashed.d	N/A	N/A	DK
Nielsen; Bjarne R.o	Virum	N/A	N/A	DK
slashed.nfeldt	Farum	N/A	N/A	DK
Nielsen; Ruby Illum				

APPL-NO: 09/ 632392

DATE FILED: August 4, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. Ser. No. 09/499,531 filed on Feb. 10, 2000, now U.S. Pat. No. 6,136,571, which is a continuation of U.S. Ser. No. 09/198,672 filed on Nov. 23, 1998, now U.S. Pat. No. 6,129,788, which is a continuation-in-part of U.S. Ser. No. 09/107,657 filed on Jun. 30, 1998, abandoned, which is a continuation-in-part of U.S. Ser. No. 08/979,673 filed on Nov. 26, 1997, the contents of which are fully incorporated herein by reference.

US-CL-CURRENT: 435/96, 127/40 , 127/55 , 435/105 , 435/98 , 435/99

ABSTRACT:

The present invention relates to a method for the production of saccharide preparations, i.e., syrups, by saccharifying a liquefied starch solution, which method comprises a saccharification step during which step one or more enzymatic saccharification stages takes place, and the subsequent steps of one or more high temperature membrane separation steps, and re-circulation of the saccharification enzyme, in which method the membrane separation steps are carried out as an integral part of the saccharification step. In another specific aspect, the invention provides a method of producing a saccharide preparation, which method comprises an enzymatic saccharification step, and the subsequent steps of one or more high temperature membrane separation steps and re-circulation of the saccharification enzyme.

10 Claims, 5 Drawing figures

Exemplary Claim Number: 1,5,8

Number of Drawing Sheets: 5

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Brief Summary Text - BSTX (21):
Isomaltooligosaccharide syrups are sometimes referred to as "Alo mixtures" and defines a mixture containing isomaltose (O-.alpha.-D-glucopyranosyl-(1-6)-D-glucopyranose), panose, isomaltotriose and several other branched oligosaccharides composed of four and five glucose residues. "Alo mixture" syrups may be produced enzymatically from starch using a thermostable bacterial .alpha.-amylase in the liquefaction step. In the following step the liquefied starch is hydrolyzed or saccharified using a .beta.-amylase and a transglucosidase simultaneously.

Brief Summary Text - BSTX (42):
It has also been found that the efficiency of a nanofiltration step, when producing dextrose, can be further increased if the purification process is carried out at an elevated temperature (i.e., of above 63.degree. C.). Moreover, it has been found that the amount of by-products formed during reaction is decreased when a glucose solution (syrup) of a lower glucose content is applied to the membrane separation step. This allow for a more efficient purification of the dextrose syrup, and dextrose preparations, of high purity can be more easily obtained. Finally, yields are improved and costs reduced by employing thermostable enzymes.

Detailed Description Text - DETX (55):
Isomaltooligosaccharide syryps or "Alo mixtures" may be produced from starch by first performing a liquefaction step using a thermostable bacterial .alpha.-amylase. The degree of hydrolysis (DE) of the starch is kept between 6-10. The liquefied starch is then subjected simultaneously to a .beta.-amylase (e.g., soybean .beta.-amylase) and a transglucosidase (e.g., from Aspergillus niger), 2-4 g and 0.2-0.3 g/kg starch, respectively, at 60.degree. C., pH 5.0, for about 72 hours. The reaction mixture is purified and concentrated to obtain the isomaltooligosaccharide product.

Detailed Description Text - DETX (75):
Thermostable Glucoamylase Enzymes

Detailed Description Text - DETX (76):
Preferably, the saccharification step of the invention is performed in presence of a thermostable glucoamylase enzyme (EC. 3.2.1.3).

Detailed Description Text - DETX (77):
Saccharification Including Using a Thermostable AMG may be Carried out as Described in Example 3.

Detailed Description Text - DETX (79):
In another preferred embodiment the glucoamylase used for the saccharification method of the invention has a residual activity higher that the wild-type A. niger AMG (SEQ ID NO: 2), determined as described in the Materials and Methods section, i.e., determined as residual activity after incubation for 30 minutes in 50 mM NaOAc, pH 4.5, 70.degree. C., 0.2 AGU/ml.

Detailed Description Text - DETX (80):
The glucoamylase enzyme may preferably be derived from a strain of Aspergillus, in particular Aspergillus niger or Aspergillus luchuenesis, a strain of Trichoderma viride, a strain of Rhizopus sp., in particular a strain of Rhizopus niveus, a strain of Endomyces sp., a strain of Cephalosporium cherticola, a strain of Clostridium, in particular Clostridium thermoamylolyticum, Clostridium thermosulphurogenes and Clostridium thermohydrosulphuricum, a strain of Pestalotiopsis, or a strain of Talaromyces.

in particular Talaromyces duponti, Talaromyces emersonii and Talaromyces thermophilus.

Detailed Description Text - DETX (82):
Thermostable De-branching Enzymes

Detailed Description Text - DETX (83):
Preferably, the saccharification step of the invention is performed in presence of a thermostable de-branching enzyme. Preferably, the de-branching enzyme is a pullulanase (EC 3.2.1.41) or an isoamylase (EC 3.2.1.68).

Detailed Description Text - DETX (84):
A thermostable pullulanase may be derived from a strain of Bacillus, in particular Bacillus naganoenis or Bacillus acidopullulyticus, a strain of Clostridium, in particular Clostridium thermosulphurogenes and Clostridium thermohydrosulphuricum, or a strain of Pyrococcus, in particular Pyrococcus woesie and Pyrococcus furiosus.

Detailed Description Text - DETX (85):
A thermostable isoamylase may be derived from a strain of Flavobacterium, in particular Flavobacterium odoratum, a strain derived from the thermophilic acrhaebacterium Sulfolobus acidocaldarius (Hayashibara, (1996) Biochimica et Biophysica Acta 1291, p. 177-181, such as Sulfolobus acidocaldarius ATCC33909 and from a strain of Rhodethermus marius.

Detailed Description Text - DETX (87):
In an embodiment, the saccharification step of the invention is performed in presence of a thermostable .alpha.-amylase, preferably a fungal .alpha.-amylase.

Detailed Description Text - DETX (91):
When using a bacterial .alpha.-amylase in the saccharification step of the invention it may suitable be performed in presence of a thermostable bacterial .alpha.-amylase, such as a Bacillus .alpha.-amylase, such as the commercially available B. licheniformis (e.g., Termamylm from Novo Nordisk) or variants thereof. Another suitable thermostable amylase is the maltogenic amylase from Bacillus stearothermophilus (e.g., Maltogenase.TM. from Novo Nordisk).

Detailed Description Text - DETX (98):
Thermostable CGTases

Detailed Description Text - DETX (99):
When producing cyclodextrins the cyclization step may preferably be performed in the presence of a thermostable CGTase. Suitable thermostable CGTases include the CGTases from the thermophilic anaerobic genus Thermoanaerobacter, the genus Bacillus, such as B. macerans, B. circulans, B.sterothermophilus, and B. subtilis.

Detailed Description Text - DETX (169):
Construction of a Thermostable AMG G2 S119P Variant Site-directed Mutagenesis

Detailed Description Text - DETX (183):
Saccharification Using a Thermostable AMG Variant to Produce Dextrose Liquor.

US-PAT-NO: 6255084

DOCUMENT-IDENTIFIER: US 6255084 B1

TITLE: Thermostable glucoamylase

DATE-ISSUED: July 3, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Nielsen; Bjarne R.o slashed.nfeldt	Virum	N/A	N/A	DK
	Farum	N/A	N/A	DK
Nielsen; Ruby Ilum	Veks.o slashed.	N/A	N/A	DK
Lehmbeck; Jan				

APPL-NO: 09/ 199290

DATE FILED: November 24, 1998

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. application Ser. No. 09/107,657, filed on Jun. 30, 1998, now abandoned which is a CIP of U.S. application Ser. No. 08/979,673, filed on Nov. 26, 1997, now abandoned and claims priority under 35 U.S.C. 119 of Danish applications 1557/97 filed on Dec. 30, 1997 and PA 1998 00925 filed on Jul. 10, 1998 and U.S. Provisionals 60/070,746 filed on Jan. 8, 1998 and 60/094,344 filed on Jul. 28, 1998, the contents of which are fully incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	1557/97	December 30, 1997
DK	1998 00925	July 10, 1998

US-CL-CURRENT: 435/96, 435/205 , 435/254.1

ABSTRACT:

The invention relates to an isolated thermostable glucoamylase derived from Talaromyces emersonii suitable for starch conversion processes.

24 Claims, 15 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

---------- KWIC ---------

Abstract Text - ABTX (1):

The invention relates to an isolated thermostable glucoamylase derived from Talaromyces emersonii suitable for starch conversion processes.

TITLE - TI (1):

Thermostable glucoamylase

Brief Summary Text - BSTX (2):
The present invention relates to a thermostable glucoamylase suitable for, e.g., starch conversion, e.g., for producing glucose from starch. The present invention also relates to the use of said thermostable glucoamylase in various processes, in particular in the saccharification step in starch convention processes.

Brief Summary Text - BSTX (8):
U.S. Pat. No. 4,247,637 describes a thermostable glucoamylase having a molecular weight of about 31,000 Da derived from Talaromyces duponti suitable for saccharifying a liquefied starch solution to a syrup. The glucoamylase is stated to retain at least about 90% of its initial glucoamylase activity when held at 70.degree. C. for 10 minutes at pH 4.5.

Brief Summary Text - BSTX (9):
U.S. Pat. No. 4,587,215 discloses a thermostable amyloglucosidase derived from the species Talaromyces thermophilus with a molecular weight of about 45,000 Da. The disclosed amyloglucosidase (or glucoamylase) loses its enzymatic activity in two distinct phases, an initial period of rapid decay followed by a period of slow decay. At 70.degree. C. (pH=5.0) the half-life for the fast decay is about 18 minutes with no measurable loss of activity within an hour in the second phase of decay. Bunni L et al., (1989), Enzyme Microb. Technol., Vol. 11, p. 370-375. concerns production, isolation and partial characterization of an extracellular amylolytic system composed of at least one form of .alpha.-amylase and one form of an .alpha.-glucosidase produced by Talaromyces emersonii CBS 814.70. Only the .alpha.-amylase is isolated, purified and characterized.

Brief Summary Text - BSTX (11):
The present invention is based upon the finding of a novel thermostable glucoamylase suitable for use, e.g., in the saccharification step in starch conversion processes.

Brief Summary Text - BSTX (14):
The inventors of the present invention have isolated, purified and characterized a thermostable glucoamylase from a strain of Talaromyces emersonii now deposited with the Centraalbureau voor Schimmelcultures under the number CBS 793.97.

Brief Summary Text - BSTX (19):
The isolated glucoamylase has a very high thermal stability in comparison to prior art glucoamylases, such as the Aspergillus niger glucoamylase (available from Novo Nordisk A/S under the trade name AMG). The T1/2 (half-life) was determined to be about 120 minutes at 70.degree. C. (pH 4.5) as described in Example 2 below. The T1/2 of the recombinant T. emersonii AMG expressed in yeast was determined to be about 110 minutes as described in Example 12.

Brief Summary Text - BSTX (28):
Finally, the invention relates to an isolated pure culture of the microorganism Talaromyces emersonii CBS 793.97 or a mutant thereof capable of producing a glucoamylase of the invention.

Drawing Description Text - DRTX (2):
FIG. 1 shows the SDS-PAGE gel (stained with Coomassie Blue) used for determining the molecular weight (M.sub.w) of the purified Talaromyces emersonii CBS 793.97 glucoamylase of the present invention.

Drawing Description Text - DRTX (6):
FIG. 2 shows the pH activity profile of Talaromyces emersonii and Aspergillus niger glucoamylase (AMG) in 0.5% maltose at 60.degree. C.;

Drawing Description Text - DRTX (7):
FIG. 3 shows the temperature activity profile of the Talaromyces emersonii CBS 793.97 glucoamylase vs. Aspergillus niger glucoamylase (AMG);

Drawing Description Text - DRTX (8):
FIG. 4 shows the curve for determining T.sub.1/2 (half-life) in 50 mM NaOAc, 0.2 AGU/ml, pH 4.5, at 70.degree. C. of Talaromyces emersonii CBS 793.97 glucoamylase vs. Aspergillus niger glucoamylase (AMG);

Drawing Description Text - DRTX (14):
FIG. 10 shows the SDS page gel of two transformants, JaL228#5.77 and HowB112#8.10, expressing the Talaromyces emersonii glucoamylase of the invention. JaL228 and HowB112 are the untransformed parent strains. MW: Promega's Protein Molecular;

Drawing Description Text - DRTX (17):
FIG. 13 shows the result of the test for determining the thermostability of recombinant Talaromyces emersonii AMG produced in yeast at 70.degree. C., pH 4.5, 0.2 AGU/ml. T1/2 was determined to about 110.degree. C.

Detailed Description Text - DETX (2):
The present invention is based upon the finding of a novel thermostable glucoamylase suitable for use in, e.g., the saccharification step in a starch conversion process.

Detailed Description Text - DETX (3):
The inventors of the present invention have isolated, purified and characterized a glucoamylase from a strain of Talaromyces emersonii CBS 793.97. The glucoamylase turned out to have a very high thermal stability in comparison to prior art glucoamylases.

Detailed Description Text - DETX (5):
T1/2 (half-life) of the isolated Talaromyces emersonii CBS 793.97 glucoamylase was determined to be about 120 minutes at 70.degree. C. as described in Example 2 below and to be about 110.degree. C. for the T. emersonii produced in yeast as described in Example 12.

Detailed Description Text - DETX (15):
Talaromyces emersonii Glucoamylase Amino Acid Sequence

Detailed Description Text - DETX (16):
The inventors have sequenced the thermostable glucoamylase derived from Talaromyces emersonii CBS 793.97 as will be described further in the Example 3 below. According to the invention the Talaromyces AMG may have a Asp145Asn (or D145N) substitution (using SEQ ID NO: 7 numbering).

Detailed Description Text - DETX (37):
The present invention provides a method of using the thermostable glucoamylase of the invention for producing glucose and the like from starch. Generally, the method includes the steps of partially hydrolyzing precursor starch in the presence of .alpha.-amylase and then further hydrolyzing the release of D-glucose from the non-reducing ends of the starch or related oligo- and polysaccharide molecules in the presence of glucoamylase by cleaving .alpha.-(1.fwdarw.4) and .alpha.-(1.fwdarw.6) glucosidic bonds.

Detailed Description Text - DETX (40):
By using a thermostable glucoamylase of the invention saccharification processes may be carried out at a higher temperature than traditional batch saccharification processes. According to the invention saccharification may be carried out at temperatures in the range from above 60-80.degree. C., preferably 63-75.degree. C. This applies both for traditional batch processes (described above) and for continuous saccharification processes.

Detailed Description Text - DETX (41):
Actually, continuous saccharification processes including one or more membrane separation steps, i.e., filtration steps, must be carried out at temperatures of above 60.degree. C. to be able to maintain a reasonably high flux over the membrane. Therefore, a thermostable glucoamylase of the invention provides the possibility of carrying out large scale continuous saccharification processes at a fair price within and period of time acceptable for industrial saccharification processes. According to the invention the saccharification time may even be shortened.

Detailed Description Text - DETX (42):
The activity of a glucoamylase of the invention is generally substantially higher at temperatures between 60.degree. C.-80.degree. C. than at the traditionally used temperature between 30-60.degree. C. Therefore, by increasing the temperature at which the glucoamylase operates the saccharification process may be carried out within a shorter period of time or the process may be carried out using lower enzyme dosage.

Detailed Description Text - DETX (44):
By using a glucoamylase with increased specific activity (measured as activity towards maltose), a lower enzyme dosage may be required in the saccharification process.

Detailed Description Text - DETX (55):
Glucoamylase derived from the deposited filamentous fungus Talaromyces emersonii CBS No. 793.97 hasbeen deposited with the Centraalbureau voor Schimmelcultures, P.O. Box 273, 3740 AG Baarn, the Netherlands, for the purposes of patent procedure on the date indicated below. CBS being an international depository under the Budapest Treaty affords permanence of the deposit in accordance with rule 9 of said treaty.

Detailed Description Text - DETX (63):
A. niger G1 glucoamylase gene is shown in SEQ ID NO: 8 T. emersonii glucoamylase gene with introns is shown in FIG. 5 and SEQ ID NO: 33. The introns are shown in FIG. 5.

Detailed Description Text - DETX (123):
Characterisation of the Talaromyces emersonii Glucoamylase

Detailed Description Text - DETX (124):
The purified Talaromyces emersonii CBS 793.97 glucoamylase was used for characterisation.

Detailed Description Text - DETX (130):
The pH-activity dependency of the Talaromyces emersonii glucoamylase was determined and compared with profile of Aspergillus niger glucoamylase.

Detailed Description Text - DETX (133):
The temperature-activity dependency of the Talaromyces emersonii glucoamylase of the invention was determined and compared with the profile of Aspergillus niger glucoamylase. 200 .mu.l 0.5% maltose, pH 4.3 was incubated

at 37, 50, 60, 70, 75, 80 and 90.degree. C. and the reaction was started by adding 10 .mu.l enzyme (0.25 AGU/ml); reaction time was 10 minutes. The result of the test is shown in FIG. 3.

Detailed Description Text - DETX (135):
The thermal stability of the Talaromyces emersonii glucoamylase was determined and compared with the thermal stability of Aspergillus niger glucoamylase. The method used is described above in the "Material and Methods" section as "Thermal Stability I (T1/2 (half-life) determination of AMG".

Detailed Description Text - DETX (136):
The T1/2 of the Talaromyces emersonii glucoamylase was determined to about 120 minutes at 70.degree. C. The T1/2 of the Aspergillus niger glucoamylase was determined to 7 minutes under the same conditions (See FIG. 4).

Detailed Description Text - DETX (140):
Sequencing of the N-terminal of T. emersonii Glucoamylase

Detailed Description Text - DETX (141):
The N-terminal amino acid sequence of T. emersonii glucoamylase was determined following SDS-PAGE and electroblotting onto a PVDF-membrane. Peptides were derived from reduced and S-carboxymethylated glucoamylase by cleaving with a lysyl-specific protease. The resulting peptides were fractionated and re-purified using RP-HPLC before subjected to N-terminal sequence determination.

Detailed Description Text - DETX (150):
The Full Length T. emersonii Glucoamylase

Detailed Description Text - DETX (151):
The full length T. emersonii glucoamylase amino acid sequence shown in SEQ ID NO: 7 was identified using standard methods.

Detailed Description Text - DETX (153):
Cloning and Sequencing of the Talaromyces emersonii Glucoamylase Gene

Detailed Description Text - DETX (202):
Expression of Talaromyces emersonii Glucoamylase in Yeast

Detailed Description Text - DETX (205):
The yeast cells were grown at 30.degree. C. for 3 days in Sc-ura medium followed by growth for 3 days in YPD. The culture was then centrifuged and the supernatant was used for the thermostability assay described in the "Materials and Method" section.

Detailed Description Text - DETX (210):
200 ml culture broth from fermentation of A. niger HowB112 harboring the Talaromyces emersonii gene was centrifuged at 9000 rpm and dialyzed against 20 mM NaOac, pH 5 over night. The solution was then applied on a S Sepharose column (200 ml) previously equilibrated in 20 mM NaOAc, pH 5. The glucoamylase was collected in the effluent, and applied on a Q Sepharose column (50 ml) previously equilibrated in 20 mM NaOAC, pH 4.5. Unbound material was washed of the column and the glucoamylase was eluted using a linear gradient from 0-0.3 M NaCl in 20 mM NaOAc over 10 column volumes. Purity of the glucoamylase fraction was checked by SDS-PAGE and only one single band was seen. The molecular weight was again found to about 70 kdal as seen for the wild type glucoamylase. The specific activity towards maltose was measured and a specific activity of 8.0 AGU/mg (37.degree. C.) and 21.0 AGU/mg (60.degree. C.) were found which is in accordance the data on the wild type enzyme.

Detailed Description Text - DETX (216):
The saccharification performance of the Talaromyces emersonii glucoamylase was tested at different temperatures with and without the addition of acid .alpha.-amylase and pullulanase. Saccharification was run under the following conditions: Substrate: 10 DE Maltodextrin, approx. 30% DS (w/w) Temperatures: 60, 65, or 70.degree. C. Initial pH: 4.5 Enzyme dosage: Recombinant Talaromyces emersonii glucoamylase produced in A. niger: 0.24 or 0.32 AGU/g DS Acid .alpha.-amylase derived from A. niger: 0.020 AFAU/g DS Pullulanase derived from Bacillus: 0.03 PUN/g DS

Detailed Description Text - DETX (224):
The thermal stability of recombinant Talaromyces emersonii glucoamylase expressed in yeast (purified using the method described in Example 9) was determined at 70.degree. C., pH 4.5, 0.2 AGU/ml using the method described above in the "Material and Methods" section as "Thermal Stability I (T1/2 (half-life) determination of AMG".

Detailed Description Text - DETX (225):
FIG. 13 shows the result of the test. The T1/2 of the recombinant Talaromyces emersonii glucoamylase expressed in yeast was determined to about 110 minutes at 70.degree. C.

Claims Text - CLTX (1):
1. An isolated enzyme with glucoamylase activity, wherein the enzyme (a) is derived from Talaromyces and has a T.sub.1/2 (half-life) of at least 100 minutes in 50 mM NaOAc, 0.2 Novo Amyloglucosidase Unit (AGU)/ml, pH 4.5, at 70.degree. C. or (b) has an amino acid sequence that has at least 80% identity with the glucoamylase of SEQ ID NO:7.

US-PAT-NO: 6136571

DOCUMENT-IDENTIFIER: US 6136571 A

TITLE: Method of producing saccharide preparations

DATE-ISSUED: October 24, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Liaw; Gin C.	Decatur	IL	N/A	N/A
Pedersen; Sven	Gentofte	N/A	N/A	DK
Hendriksen; Hanne Vang	Holte	N/A	N/A	DK
Svendsen; Allan	Birker.o slashed.d	N/A	N/A	DK
Nielsen; Bjarne R.o	Virum	N/A	N/A	DK
slashed.nfeldt	Farum	N/A	N/A	DK
Nielsen; Ruby Illum				

APPL-NO: 09/ 499531

DATE FILED: February 10, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. Ser. No. 09/198,672 filed on Nov. 23, 1998, which is a continuation-in-part of U.S. Ser. No. 09/107,657 filed on Jun. 30, 1998, now abandoned, which is a continuation-in-part of U.S. Ser. No. 08/979,673 filed on Nov. 26, 1997, now abandoned, the contents of which are fully incorporated herein by reference.

US-CL-CURRENT: 435/96, 127/40 , 127/55 , 435/98 , 435/99

ABSTRACT:

The present invention relates to a method for the production of saccharide preparations, i.e., syrups, by saccharifying a liquefied starch solution, which method comprises a saccharification step during which step one or more enzymatic saccharification stages takes place, and the subsequent steps of one or more high temperature membrane separation steps, and re-circulation of the saccharification enzyme, in which method the membrane separation steps are carried out as an integral part of the saccharification step. In another specific aspect, the invention provides a method of producing a saccharide preparation, which method comprises an enzymatic saccharification step, and the subsequent steps of one or more high temperature membrane separation steps and re-circulation of the saccharification enzyme.

15 Claims, 5 Drawing figures

Exemplary Claim Number: 1,8

Number of Drawing Sheets: 5

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Brief Summary Text - BSTX (22):
Isomaltooligosaccharide syrups are sometimes referred to as "Alo mixtures" and defines a mixture containing isomaltose (O-.alpha.-D-glucopyranosyl-(1-6)-D-glucopyranose), panose, isomaltotriose and several other branched oligosaccharides composed of four and five glucose residues. "Alo mixture" syrups may be produced enzymatically from starch using a thermostable bacterial .alpha.-amylase in the liquefaction step. In the following step the liquefied starch is hydrolyzed or saccharified using a .beta.-amylase and a transglucosidase simultaneously.

Brief Summary Text - BSTX (43):
It has also been found that the efficiency of a nanofiltration step, when producing dextrose, can be further increased if the purification process is carried out at an elevated temperature (i.e., of above 63.degree. C.). Moreover, it has been found that the amount of by-products formed during reaction is decreased when a glucose solution (syrup) of a lower glucose content is applied to the membrane separation step. This allow for a more efficient purification of the dextrose syrup, and dextrose preparations, of high purity can be more easily obtained. Finally, yields are improved and costs reduced by employing thermostable enzymes.

Detailed Description Text - DETX (56):
Isomaltooligosaccharide syryps or "Alo mixtures" may be produced from starch by first performing a liquefaction step using a thermostable bacterial .alpha.-amylase. The degree of hydrolysis (DE) of the starch is kept between 6-10. The liquefied starch is then subjected simultaneously to a .beta.-amylase (e.g., soybean .beta.-amylase) and a transglucosidase (e.g., from Aspergillus niger), 2-4 g and 0.2-0.3 g/kg starch, respectively, at 60.degree. C., pH 5.0, for about 72 hours. The reaction mixture is purified and concentrated to obtain the isomaltooligosaccharide product.

Detailed Description Text - DETX (76):
Thermostable Glucoamylase Enzymes

Detailed Description Text - DETX (78):
presence of a thermostable glucoamylase enzyme (EC. 3.2.1.3).

Detailed Description Text - DETX (79):
Saccharification including using a thermostable AMG may be carried out as described in Example 3.

Detailed Description Text - DETX (81):
In another preferred embodiment the glucoamylase used for the saccharification method of the invention has a residual activity higher that the wild-type A. niger AMG (SEQ ID NO: 2), determined as described in the Materials and Methods section, i.e., determined as residual activity after incubation for 30 minutes in 50 mM NaOAc, pH 4.5, 70.degree. C., 0.2 AGU/ml.

Detailed Description Text - DETX (82):
The glucoamylase enzyme may preferably be derived from a strain of Aspergillus, in particular Aspergillus niger or Aspergillus luchuenesis, a strain of Trichoderma viride, a strain of Rhizopus sp., in particular a strain of Rhizopus niveus, a strain of Endomyces sp., a strain of Cephalosporium cherticola, a strain of Clostridium, in particular Clostridium thermoamylolyticum, Clostridium thermosulphurogenes and Clostridium thermohydrosulphuricum, a strain of Pestalotiopsis, or a strain of Talaromyces, in particular Talaromyces duponti, Talaromyces emersonii and Talaromyces thermophilus.

Detailed Description Text - DETX (84):
Thermostable De-branching Enzymes

Detailed Description Text - DETX (85):
Preferably, the saccharification step of the invention is performed in presence of a thermostable de-branching enzyme. Preferably, the de-branching enzyme is a pullulanase (EC 3.2.1.41) or an isoamylase (EC 3.2.1.68).

Detailed Description Text - DETX (86):
A thermostable pullulanase may be derived from a strain of Bacillus, in particular Bacillus naganoenis or Bacillus acidopullulyticus, a strain of Clostridium, in particular Clostridium thermosulphurogenes and Clostridium thermohydrosulphuricum, or a strain of Pyrococcus, in particular Pyrococcus woesie and Pyrococcus furiosus.

Detailed Description Text - DETX (87):
A thermostable isoamylase may be derived from a strain of Flavobacterium, in particular Flavobacterium odoratum, a strain derived from the thermophilic acrhaebacterium Sulfolobus acidocaldarius (Hayashibara, (1996) Biochimica et Biophysica Acta 1291, p. 177-181, such as Sulfolobus acidocaldarius ATCC33909 and from a strain of Rhodethermus marius.

Detailed Description Text - DETX (89):
In an embodiment, the saccharification step of the invention is performed in presence of a thermostable .alpha.-amylase, preferably a fungal .alpha.-amylase.

Detailed Description Text - DETX (93):
When using a bacterial .alpha.-amylase in the saccharification step of the invention it may suitable be performed in presence of a thermostable bacterial .alpha.-amylase, such as a Bacillus .alpha.-amylase, such as the commercially available B. licheniformis (e.g., Termamyl.TM. from Novo Nordisk) or variants thereof. Another suitable thermostable amylase is the maltogenic amylase from Bacillus stearothermophilus (e.g., Maltogenase.TM. from Novo Nordisk).

Detailed Description Text - DETX (100):
Thermostable CGTases

Detailed Description Text - DETX (101):
When producing cyclodextrins the cyclization step may preferably be performed in the presence of a thermostable CGTase. Suitable thermostable CGTases include the CGTases from the thermophilic anaerobic genus Thermoanaerobacter, the genus Bacillus, such as B. macerans, B. circulans, B.sterothermophilus, and B. subtilis.

Detailed Description Text - DETX (180):
Construction of a Thermostable AMG G2 S119P Variant Site-directed Mutagenesis

Detailed Description Text - DETX (192):
Saccharification Using a Thermostable AMG Variant to Produce Dextrose Liquor

US-PAT-NO: 6129788

DOCUMENT-IDENTIFIER: US 6129788 A

TITLE: Method of producing saccharide preparations

DATE-ISSUED: October 10, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Liaw; Gin C.	Decatur	IL	N/A	N/A
Pedersen; Sven	Gentofte	N/A	N/A	DK
Hendriksen; Hanne Vang	Holte	N/A	N/A	DK
Svendsen; Allan	Birker.o slashed.d	N/A	N/A	DK
Nielsen; Bjarne R.o	Virum	N/A	N/A	DK
slashed.nfeldt	Farum	N/A	N/A	DK
Nielsen; Ruby Illum				

APPL-NO: 09/ 198672

DATE FILED: November 23, 1998

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. application Ser. No. 09/107,657 filed on Jun. 30, 1998, now abandoned, which is a continuation of Ser. No. 08/979,673 filed on Nov. 26, 1997, now abandoned, the contents of which are fully incorporated herein by reference.

US-CL-CURRENT: 127/40, 435/105 , 435/96 , 435/98

ABSTRACT:

The present invention relates to a method for the production of saccharide preparations, i.e., syrups, by saccharifying a liquefied starch solution, which method comprises a saccharification step during which step one or more enzymatic saccharification stages takes place, and the subsequent steps of one or more high temperature membrane separation steps, and recirculation of the saccharification enzyme, in which method the membrane separation steps are carried out as an integral part of the saccharification step.

16 Claims, 5 Drawing figures

Exemplary Claim Number: 1,9

Number of Drawing Sheets: 5

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Brief Summary Text - BSTX (21):

Isomaltooligosaccharide syrups are sometimes referred to as "Alo mixtures" and defines a mixture containing isomaltose (O-(.alpha.-D-glucopyranosyl-(1-6)-D-glucopyranose), panose, isomaltotriose and several other branched oligosaccharides composed of four and five glucose

residues. "Alo mixture" syrups may be produced enzymatically from starch using a thermostable bacterial .alpha.-amylase in the liquefaction step. In the following step the liquefied starch is hydrolyzed or saccharified using a .beta.-amylase and a transglucosidase simultaneously.

Brief Summary Text - BSTX (42):
It has also been found that the efficiency of a nanofiltration step, when producing dextrose, can be further increased if the purification process is carried out at an elevated temperature (i.e., of above 63.degree. C.). Moreover, it has been found that the amount of by-products formed during reaction is decreased when a glucose solution (syrup) of a lower glucose content is applied to the membrane separation step. This allow for a more efficient purification of the dextrose syrup, and dextrose preparations, of high purity can be more easily obtained. Finally, yields are improved and costs reduced by employing thermostable enzymes.

Detailed Description Text - DETX (56):
Isomaltooligosaccharide syryps or "Alo mixtures" may be produced from starch by first performing a liquefaction step using a thermostable bacterial .alpha.-amylase. The degree of hydrolysis (DE) of the starch is kept between 6-10. The liquefied starch is then subjected simultaneously to a .beta.-amylase (e.g., soybean .beta.-amylase) and a transglucosidase (e.g., from Aspergillus niger), 2-4 g and 0.2-0.3 g/kg starch, respectively, at 60.degree. C., pH 5.0, for about 72 hours. The reaction mixture is purified and concentrated to obtain the isomaltooligosaccharide product.

Detailed Description Text - DETX (76):
Thermostable Glucoamylase Enzymes

Detailed Description Text - DETX (77):
Preferably, the saccharification step of the invention is performed in presence of a thermostable glucoamylase enzyme (EC. 3.2.1.3). Saccharification including using a thermostable AMG may be carried out as described in Example 3.

Detailed Description Text - DETX (79):
In another preferred embodiment the glucoamylase used for the saccharification method of the invention has a residual activity higher that the wild-type A. niger AMG (SEQ ID NO: 2), determined as described in the Materials and Methods section, i.e., determined as residual activity after incubation for 30 minutes in 50 mM NaOAc, pH 4.5, 70.degree. C., 0.2 AGU/ml.

Detailed Description Text - DETX (83):
Thermostable De-branching Enzymes

Detailed Description Text - DETX (84):
Preferably, the saccharification step of the invention is performed in presence of a thermostable de-branching enzyme. Preferably, the de-branching enzyme is a pullulanase (EC 3.2.1.41) or an isoamylase (EC 3.2.1.68).

Detailed Description Text - DETX (85):
A thermostable pullulanase may be derived from a strain of Bacillus, in particular Bacillus naganoenis or Bacillus acidopullulyticus, a strain of Clostridium, in particular Clostridium thermosulphurogenes and Clostridium thermohydrosulphuricum, or a strain of Pyrococcus, in particular Pyrococcus woesie and Pyrococcus furiosus.

Detailed Description Text - DETX (86):
A thermostable isoamylase may be derived from a strain of Flavobacterium, in

particular Flavobacterium odoratum, a strain derived from the thermophilic acrhaebacterium Sulfolobus acidocaldarius (Hayashibara, (1996) Biochimica et Biophysica Acta 1291, p. 177-181, such as Sulfolobus acidocaldarius ATCC33909 and from a strain of Rhodethermus marius.

Detailed Description Text - DETX (88):
In an embodiment, the saccharification step of the invention is performed in presence of a thermostable .alpha.-amylase, preferably a fungal .alpha.-amylase.

Detailed Description Text - DETX (92):
When using a bacterial .alpha.-amylase in the saccharification step of the invention it may suitable be performed in presence of a thermostable bacterial .alpha.-amylase, such as a Bacillus .alpha.-amylase, such as the commercially available B. licheniformis (e.g., Termamyl.TM. from Novo Nordisk) or variants thereof. Another suitable thermostable amylase is the maltogenic amylase from Bacillus stearothermophilus (e.g., Maltogenase.TM. from Novo Nordisk).

Detailed Description Text - DETX (99):
Thermostable CGTases

Detailed Description Text - DETX (100):
When producing cyclodextrins the cyclization step may preferably be performed in the presence of a thermostable CGTase. Suitable thermostable CGTases include the CGTases from the thermophilic anaerobic genus Thermoanaerobacter, the genus Bacillus, such as B. macerans, B. circulans, B. sterothermophilus, and B. subtilis.

Detailed Description Text - DETX (168):
Construction of a Thermostable AMG G2 S119P Variant Site-Directed Mutagenesis

Detailed Description Text - DETX (181):
Saccharification Using a Thermostable AMG Variant to Produce Dextrose Liquor.

US-PAT-NO: 6087149

DOCUMENT-IDENTIFIER: US 6087149 A

TITLE: Starch conversion process

DATE-ISSUED: July 11, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tsutsumi; Noriko	Chiba-ken	N/A	N/A	JP
Bisg.ang.rd-Frantzen; Henrik	Bagsv.ae butted.rd	N/A	N/A	DK
Svendsen; Allan	Birker.o slashed.d	N/A	N/A	DK

APPL-NO: 09/ 129075

DATE FILED: August 4, 1998

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of PCT/DK98/00304 filed Jul. 2, 1998 which claims priority under 35 U.S.C. 119 of Danish application 0787/97 filed Jul. 2, 1997 and U.S. provisional application Ser. No. 60/055,867 filed Aug. 13, 1997, the contents of which are fully incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	0787/97	February 7, 1997

US-CL-CURRENT: 435/210, 435/252.3 , 435/252.31 , 435/252.33 , 435/254.11 , 435/254.2 , 435/254.21 , 435/254.22 , 435/254.23 , 435/254.3 , 435/254.6 , 435/254.7 , 435/320.1 , 435/325 , 536/23.1 , 536/23.2

ABSTRACT:

The present invention relates to a starch conversion process of the type which includes a debranching step wherein an isoamylase being active at the process conditions prevailing is used for debranching the starch and to the use of thermostable isoamylases for starch conversion. The invention further relates to an isolated isoamylase obtained from a strain of the genus Rhodothermus and to cloned DNA sequences encoding isoamylases derived from a strain of Rhodothermus or Sulfolobus, to expression vectors comprising said DNA sequence, host cells comprising such expression vectors, and finally to methods for producing said isoamylases.

23 Claims, 7 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 7

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Abstract Text - ABTX (1):
The present invention relates to a starch conversion process of the type which includes a debranching step wherein an isoamylase being active at the process conditions prevailing is used for debranching the starch and to the use of thermostable isoamylases for starch conversion. The invention further relates to an isolated isoamylase obtained from a strain of the genus Rhodothermus and to cloned DNA sequences encoding isoamylases derived from a strain of Rhodothermus or Sulfolobus, to expression vectors comprising said DNA sequence, host cells comprising such expression vectors, and finally to methods for producing said isoamylases.

Brief Summary Text - BSTX (2):
The invention relates to a starch conversion process of the type which includes a debranching step. The invention also relates to the use of a thermostable isoamylase for debranching starch. The invention further relates to an isolated isoamylase obtained from a strain of the genus Rhodothermus, to cloned DNA sequences encoding isoamylases derived from a strain of Rhodothermus or Sulfolobus, to expression vectors comprising said DNA sequence, host cells comprising such expression vectors, and finally to methods for producing said isoamylases.

Brief Summary Text - BSTX (32):
It is the object of the present invention to provide a starch conversion process of the type which includes a debranching step which results in a reduced formation of the undesired trisaccharide panose. The invention also relates to a novel thermostable isoamylase suitable for use in the starch conversion process of the invention.

Brief Summary Text - BSTX (36):
In the second aspect the invention relates to the use of a thermostable isoamylase in starch conversion processes.

Detailed Description Text - DETX (2):
It is the object of the present invention to provide a starch conversion process of the type which includes a debranching step which results in a reduced formation of the undesired trisaccharide panose. The invention also relates to a novel thermostable isoamylase suitable for use in the starch conversion process of the invention.

Detailed Description Text - DETX (7):
A thermostable isoamylase makes it possible to perform the liquefaction and the debranching at the same time before the saccharification step.

Detailed Description Text - DETX (8):
Specific examples of thermostable debranching enzymes are the thermostable isoamylases derived from the thermophilic bacteria such as Sulfolobus acidocaldarius ATCC 33909 (Maruta, K. et al., Biochimica et Biophysica Acta 1291, p. 177-181 (1996)), Sulfolobus solfataricus ATCC 35092 (accession number: Y08256) and Rhodothermus marinus DSM 4252 as will be described further below.

Detailed Description Text - DETX (12):
In a preferred embodiment the debranching enzyme being active at the process conditions prevailing is a thermostable isoamylase.

Detailed Description Text - DETX (18):
Isoamylases which can be used according to the invention include the thermostable isoamylase derived from the thermophilic archaebacteria Sulfolobus acidocaldarius, Sulfolobus solfataricus and the thermophilic eubacterium Rhodothermus marinus (as will be described in details below).

Detailed Description Text - DETX (24):
In the second aspect the invention relates to the use of a thermostable isoamylase in starch conversion processes, including fructose syrup conversion processes and for producing fat replacers.

Detailed Description Text - DETX (25):
In the case of the starch conversion process is a starch depolymerization process the thermostable isoamylase is used in combination with an .alpha.-amylase during the liquefaction step. The thermostable isoamylase may be derived from the thermophilic archaebacteria Sulfolobus acidocaldarius or Sulfolobus solfataricus or from Rhodothermus marinus.

Detailed Description Text - DETX (26):
The thermostable isoamylase may be used during the liquefaction step of a starch to glucose syrup or fructose syrup conversion process. The thermostable isoamylase may also be used in processes for producing fat replacers from starch.

Detailed Description Text - DETX (29):
Several advantages are obtained by the addition of a thermostable isoamylase.

Detailed Description Text - DETX (31):
As the debranching enzyme is a thermostable isoamylase the debranching by a pullulanase during saccharification can be left out. This is advantageous as pullulanase tends to condense maltose into a panose precursor, 6.sup.2 -.alpha.-maltosylmaltose which is hydrolysed into panose by glucoamylase.

Detailed Description Text - DETX (32):
By prolonging the liquefaction step the DE is increased from 10-15 to e.g. 15-20 reducing the need for glucoamylase (e.g. AMG.TM.). This reduced glucoamylase requirement is advantageous as the formation of isomaltose is reduced. Isomaltose is formed from D-glucose resulting from depolymerization of linear oligosaccharides by glucoamylase which removes D-glucose from the non-reducing chain-ends. All together less glucoamylase activity results in an increased glucose yield.

Detailed Description Text - DETX (38):
When using a thermostable isoamylase up front in the liquefaction process the process is less dependent of the specificity of the .alpha.-amylase and the formation of the panose precursors. This change allows an increased process time for the liquefaction and a higher DX than the normal value of DX 10-12 is obtained. If a more intensive liquefaction is allowed and a higher DX value is obtained by the use of a thermostable debranching enzyme its possible to increase the concentration of Dry Substance (DS) from the normal 30-35% to a higher percentage. Such an increase in %DS is advantageous as the evaporation costs are reduced significantly downstream in the High Fructose Corn Syrup (HFCS) process.

Detailed Description Text - DETX (39):
How to Identify Suitable Thermostable Isoamylases

Detailed Description Text - DETX (40):
Suitable thermostable isoamylases may be identified as described in Example 1 by first identifying conserved regions of amino acid sequence by aligning isoamylase sequences available. On the basis of the conserved regions PCR primers are designed. Genomic DNA stocks of a number of bacterial strains are then subjected to PCR, and strains yielding a fragment of the expected size are

selected. The fragments are purified, sequenced, and aligned to confirm the homology with published isoamylase sequences. Among the strains identified, ones with the highest optimum growth temperature are further selected.

Detailed Description Text - DETX (59):
Characterisation of the Novel Thermostable Isoamylase from Rhodothermus marinus

Detailed Description Text - DETX (203):
To provide thermostable isoamylases an alignment of five known isoamylases of which the protein sequence data is available was made (see Table 1).

Detailed Description Text - DETX (229):
Cloning of a Gene Encoding a Thermostable Isoamylase and its Expression in E. coli

US-PAT-NO: 4888285

DOCUMENT-IDENTIFIER: US 4888285 A

TITLE: Enzyme immobilization on a water-insoluble amino group-containing carrier

DATE-ISSUED: December 19, 1989

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Nishimura; Yuusaku	Hitachi	N/A	N/A	JP
Ishida; Masahiko	Hitachi	N/A	N/A	JP
Haga; Ryoichi	Hitachi	N/A	N/A	JP

APPL-NO: 07/ 129163

DATE FILED: December 7, 1987

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	61-290432	December 8, 1986

US-CL-CURRENT: 435/176, 435/177 , 435/178

ABSTRACT:

An immobilized enzyme having high activity and stability is obtained by immobilizing an enzyme on a water-insoluble amino group-containing carrier by use of a polyfunctional cross-linking agent such as glutaraldehyde in the presence of a phenolic carboxylic acid having one or more hydroxyl groups such as tannic acid. In addition to the phenolic carboxylic acid, a basic polysaccharide such as chitosan may also be present. The amino group-containing carrier may be aminated silica gel, aminated porous glass, aminated zeolite, or water insoluble crosslinked chitosen.

20 Claims, 4 Drawing figures

Exemplary Claim Number: 12

Number of Drawing Sheets: 2

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Drawing Description Text - DRTX (4):
FIG. 3 is a graph showing the thermostability of carriers having glucoamylase immobilized thereon which were obtained by various methods.

Detailed Description Text - DETX (25):
FIG. 1 shows the relationship between glutaraldehyde concentration and the activity of an immobilized product in the case where glucoamylade was immobilized on aminated silica gel by using a polyfunctional crosslinking agent glutaraldehyde. When tannic acid which is one of the phenolic carboxylic acids is present in the immobilization reaction, an immobilized product having high activity can be obtained in a wide glutaraldehyde concentration range. On the other hand, when tannic acid is absent, the activity of immobilized product is

seriously affected by the glutaraldehyde concentration, and the optimum glutaraldehyde concentration range is very narrow. In addition, the activity of immobilized product is sharply lowered with an increase of the glutaraldehyde concentration. As it generally known, most enzymes are denatured by organic solvents to be deactivated. The reason why the activity of the immobilized product obtained in the absence of tannic acid is low is that glucoamylase is deactivated by glutaraldehyde. From the above, it can be seen that in this invention, the optimum glutaraldehyde concentration is stoichiometrical with regard to the amino groups of a carrier, as shown by the formula (1) mentioned hereinbefore. For preventing the deactivation of glucoamylase by glutaraldehyde, it was sufficient that tannic acid was present in a very small amount of, preferably about 0.1 per cent by weight, more preferable several per cent by weight based on the weight of the glucoamylase-containing aqueous solution. It was found that when the covalent binding reaction of an aminated carrier with glucoamylase by use of glutalaldehyde was carried out in the presence of tannic acid, the enzyme was not deactivated and even when a sufficient amount of glutaraldehyde was used, the reaction proceeded rapidly and completely, so that there could be obtained a carrier having glucoamylase immobilyzed thereon which had high activity and stability.

Detailed Description Text - DETX (30):

FIG. 3 shows the thermostability of carriers having glucoamylase immobilized thereon. The activity of the immobilized products obtained by use of a phenolic carboxylic acid having one or more hydroxyl groups such as tannic acid or catechol is high. But employment of a phenolic carboxylic acid having one or more hydroxyl groups alone is not desirable because glucoamylase adsorbed thereon is merely deposited on the surface of a carrier, so that glucoamylase is easily released by longtime heat-treatment, resulting in a rapid lowering of the residual activity.

Detailed Description Text - DETX (31):

On the other hand, in the case of a conventional immobilized product obtained by use of glutaraldehyde alone, glucoamylase is immobilized by covalent binding to the amino groups of a carrier, so that glucoamylase is not easily released, and its deactivation mainly accompanies thermal denaturation and the lowering of the residual activity by heat treatment is appreciably lessened. On the other hand, in the case of the immobilized product of this invention obtained by use of a phenolic carboxylic acid having one or more hydroxyl groups and glutaraldehyde, the lowering of the residual activity is still slighter, indicating that this immobilized product is excellent in thermostability.

Detailed Description Text - DETX (36):

In addition, when tannic acid alone is used together with glutaraldehyde, the environment of enzyme is an acidic atmosphere, while when a combination of chitosan and tannic acid is used together with glutaraldehyde, the environment of enzyme becomes a neutral atmosphere and this condition seems to have a beneficial effect on the thermostability of immobilized glucoamylase. Although the adding amount of chitosan depends on the immobilization conditions, a sufficient effect can be obtained when the adding amount is about 0.1% by weight based on the weight of the glucoamylase-containing aqueous solution.

Detailed Description Text - DETX (65):

The initial activities and the half lives of activity in Examples 2, 3, 4 and 5 and Comparative Examples 2, 3, 4 and 5 are shown in Table 1. It can be seen from Table 1 that when an enzyme is immobilized on an amino group-containing carrier by covalent binding by using a polyfunctional crosslinking agent glutaraldehyde, the presence of tannic acid is effective in

improving the initial activity and thermostability of the immobilized enzyme.

US-PAT-NO: 4628031

DOCUMENT-IDENTIFIER: US 4628031 A

TITLE: Thermostable starch converting enzymes

DATE-ISSUED: December 9, 1986

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Zeikus; Joseph G.	Okemos	MI	N/A	N/A
Hyun; Hyung-Hwan	Madison	WI	N/A	N/A

APPL-NO: 06/ 652586

DATE FILED: September 18, 1984

US-CL-CURRENT: 435/205, 435/162 , 435/210 , 435/842 , 435/96 , 435/98

ABSTRACT:

A thermostable pullulanase and a thermostable glucoamylase are produced by Clostridium thermohydrosulfuricum. Methods of producing the enzymes and using them to hydrolyze starch are also disclosed.

3 Claims, 12 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

---------- KWIC ----------

Abstract Text - ABTX (1):
A thermostable pullulanase and a thermostable glucoamylase are produced by Clostridium thermohydrosulfuricum. Methods of producing the enzymes and using them to hydrolyze starch are also disclosed.

TITLE - TI (1):
Thermostable starch converting enzymes

Brief Summary Text - BSTX (2):
The present invention relates to enzymes. More particularly, it relates to thermostable starch converting enzymes.

Brief Summary Text - BSTX (4):
A variety of industries (i.e., food, chemical, detergent, textile) employ microbial amylolytic enzymes to convert starch into different sugar solutions. High value is placed on the thermostability and thermoactivity of enzymes for use in the bioprocessing of starch into maltose, glucose, fructose and various sugar syrups.

Brief Summary Text - BSTX (7):
Commercial starch saccharification processes are usually operated at about 60.degree. C. to promote substrate solubility and to prevent interference by the growth of microorganisms. Both the known glucoamylases and the known

pullulanases, however, are unstable at temperatures above 60.degree. C. Thus, the practical utility of known pullulanases or glucoamylases are somewhat restricted due to their thermal instability. Therefore, the discovery of an inexpensive source of active, thermostable glucoamylase and/or pullulanase would be an important contribution to the starch processing industry.

Brief Summary Text - BSTX (8):
To date, essentially, nothing is known about the biochemical attributes of thermophilic bacteria that actively ferment starch to ethanol at temperatures of about 60.degree. C. or higher. From our earlier work we do know, however, that thermoanaerobic bacteria can often process at faster metabolic rates and produce more thermostable enzymes than mesophilic microorganisms.

Brief Summary Text - BSTX (10):
We recently discovered that the microorganism Clostridium thermohydrosulfuricum produces both a glucoamylase and a pullulanase which have unique and valuable thermoactive and thermostable characteristics.

Brief Summary Text - BSTX (11):
The enzymes are produced by culturing thermostable glucoamylase and pullulanase producing Clostridium thermohydrosulfuricum on a nutrient broth under anaerobic conditions until enzymatic activity is detectable and thereafter isolating the thermostable glucoamylase and pullulanase by conventional means.

Detailed Description Text - DETX (2):
In the preferred practice of the present invention, a thermostable glucoamylase and/or thermostable pullulanase producing Clostridium thermohydrosulfuricum having the identifying characteristics of ATCC 33223 is cultured in a nutrient medium containing starch and essential vitamins, minerals and growth factors until substantial enzymatic activity is detectable and then the enzymes are isolated by conventional means.

Detailed Description Text - DETX (27):
The extremely high temperature values observed for optimal enzyme activity of the pullulanase (85.degree. C.) and the glucoamylase (75.degree. C.) are unusual. The reported temperature optima of pullulanse and isoamylases obtained from plants or other microorganisms are below 60.degree. C. The temperature optima of glucoamylases generally fall in the range of 40.degree. to 60.degree. C., except for Humicola lanuginosa glucoamylase II with optimal activity near 65.degree. C. The extreme thermal stability of the pullulanase (up to 85.degree. C.) and glucoamylase (up to 75.degree. C.) are perhaps the highest values reported for these types of enzymes. The pullulanases from Aerobacter and Bacillus species are stable below 50.degree. C. while enzymes from Streptococcus and Streptomycete species are stable below 40.degree. C. Most of the known glucoamylases are unstable above 60.degree. C. Therefore, both the pullulanase and the glucoamylase of C. thermohydrosulfuricum are outstanding in terms of thermostability and activity at high temperatures.

Detailed Description Text - DETX (28):
The optimal pH for enzyme activity and the pH range for enzyme stability of C. thermohydrosulfuricum glucoamylase and pullulanase are in the general range reported for other sources of these enzymes. For example, the pH optima for most glucoamylases and pullulanases are 4.5 to 5.4 and 5.0 and 5.8, respectively. Likewise, pullulanase and glucoamylase from other sources are also stable between 4.5-5.5 and 5.0-6.0, respectively. Notably, both the pullulanase and glucoamylase of C. thermohydrosulfuricum display high activity and stability in similar temperature and pH ranges.

Detailed Description Text - DETX (30):
It will be appreciated by those skilled in the art that enzyme yield may be enhanced through mutation of the organism or genetic recombination techniques. Therefore, the scope of the invention should not be limited to the specific strain of organism described above because any organism capable of producing a thermostable glucoamylase and/or pullulanase similar to those produced by C. thermohydrosulfuricum having the identifying characteristics of ATCC 33223 can be used.

Claims Text - CLTX (1):
1. A method of producing a thermostable glucoamylase and a thermostable pullulanase which comprises anaerobically culturing a thermostable glucoamylase and pullulanase producing Clostridium thermohydrosulfuricum in a nutrient medium until substantial enzymatic activity is detectable and thereafter isolating the glucoamylase and pullulanase.

Claims Text - CLTX (2):
2. A thermostable glucoamylase from Clostridium thermohydrosulfuricum having the following physiochemical properties:

Claims Text - CLTX (10):
3. A thermostable pullulanase from Clostridium thermohydrosulfuricum having the following physiochemical properties:

US-PAT-NO: 4591560

DOCUMENT-IDENTIFIER: US 4591560 A

TITLE: Process for saccharification of starch using enzyme produced by fungus belonging to genus Chalara

DATE-ISSUED: May 27, 1986

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kainuma; Keiji	Sakura	N/A	N/A	JP
Kobayashi; Shoichi	Sakura	N/A	N/A	JP

APPL-NO: 06/ 566499

DATE FILED: December 29, 1983

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	58-5564	January 17, 1983

US-CL-CURRENT: 435/96, 435/203 , 435/205 , 435/911 , 435/99

ABSTRACT:

A process for the saccharification of starch, which comprises saccharifying a raw and/or gelatinized starch by the use of an amylase produced by a fungus belonging to genus Chalara to produce glucose.

According to the process of the present invention, the starch is directly saccharified, and glucose can be obtained efficiently.

10 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

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Brief Summary Text - BSTX (5):

About 2,000 strains of microorganisms living in soil and on wood were isolated by us and examined to find those microorganisms satisfying the requirement that the ratio of the gelatinized starch-hydrolyzing degree to the raw starch-hydrolyzing degree is 10:1 or less. It has been found that some microorganisms satisfy the foregoing requirement. They are strains belonging to genus Chalara, and the properties of the enzymes secreted by them are similar to those of glucoamylases in respect of the mechanisms of enzyme reactions. These enzymes are active and stable in a slightly acidic region and have greatly higher raw starch-hydrolyzing activity compared with conventional glucoamylases; that is, the ratio of the gelatinized starch-hydrolyzing activity to the raw starch-hydrolyzing activity is from 3.5:1 to 5:1, which is greatly higher compared with those of the known glucoamylases. Hence it has been found that the saccharification of raw starch can be performed advantageously on a commercial scale by using the above-described enzymes.

Claims Text - CLTX (1):

1. A process for the saccharification of starch to produce glucose, which comprises contacting a starch with an amylase produced by a fungus belonging to the genus Chalara to produce glucose in an aqueous medium having a pH of from 3 to 9.5 at a temperature of from 30.degree. to 50.degree. C. to produce said glucose, and recovering said glucose, said amylase has a pH stability of 3-9.5, an optimum temperature of 45.degree. C. for raw starch and of 50.degree. C. for gelatinized starch and thermostability of 45.degree. C.

Claims Text - CLTX (9):

9. A process for producing an amylase which has high activity in digesting starch to form glucose comprising cultivating Chalara paradoxa PNS-80 (FERM BP-422) in a culture medium containing a nitrogen source under aerobic conditions at a pH of 4-8.5 and at a temperature of 25.degree.-40.degree. C. to produce said amylase, said amylase has a pH stability of 3-9.5, an optimum temperature of 45.degree. C. for raw starch and of 50.degree. C. for gelatinized starch and thermostability of 45.degree. C.

US-PAT-NO: RE32153

DOCUMENT-IDENTIFIER: US RE32153 E

TITLE: Highly thermostable glucoamylaseand process for its production

DATE-ISSUED: May 20, 1986

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tamura; Masaki	Kamakura	N/A	N/A	JP
Shimizu; Mizuho	Hino	N/A	N/A	JP
Tago; Minoru	Tokyo	N/A	N/A	JP

APPL-NO: 06/ 761930

DATE FILED: August 2, 1985

REISSUE-DATA:

US-PAT-NO	DATE-ISSUED	APPL-NO	DATE-FILED
04247637	January 27, 1981	055723	July 9, 1979

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	53-106354	September 1, 1978

US-CL-CURRENT: 435/96, 435/205 , 435/911

ABSTRACT:

Process for the production of a thermostable glucoamylase by a strain of Talaromyces and the glucoamylase produced thereby.

8 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

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Abstract Text - ABTX (1):

Process for the production of a thermostable glucoamylase by a strain of Talaromyces and the glucoamylase produced thereby.

Brief Summary Text - BSTX (7):

A microbial strain has been discovered belonging to the genus Talaromyces which produces a glucoamylase having an optimum reaction temperature of 75.degree. C. and characterized as being capable of retaining at least about 90% of its initial glucoamylase activity when held at 70.degree. C. and pH 4.5 for 10 minutes. This invention includes the method for the production of this glucoamylase wherein a microorganism of the genus Talaromyces, which produces the glucoamylase, is cultured in a medium and the enzyme is recovered from the culture broth.

Detailed Description Text - DETX (53):

The strain, Talaromyces .[.duponti.]. .ladd.leycettanus .laddend.G45-632, is one of the embodiments of the microorganism used in this invention and any microorganism belonging to genus Talaromyces which is capable of producing the above-mentioned novel thermophilic glucoamylase can be employed as well as the strain G45-632 and its mutant strains.

Detailed Description Text - DETX (64):

A liquid culture medium consisting of 5% soluble starch, 2% corn steep liquor, 0.5% cottonseed meal, 0.5% yeast extract, 0.1% dipotassium phosphate, 0.05% magnesium sulfate and 0.01 calcium chloride was adjusted to pH 7.0 and 100 ml of this was placed in a 500 ml Erlenmeyer flask. This medium was sterilized at 121.degree. C. for 20 minutes, inoculated with Talaromyces .[.duponti.]. .ladd.leycettanus .laddend.strain G45-632 and incubated at 40.degree. C. for 7 days on a shaker. After the culture was completed, the mycelia were removed from the culture fluid by filtration. The filtrate was found to contain 60 units of glucoamylase activity per milliliter.

Detailed Description Paragraph Table - DETL (1):

TABLE I

COMPARISON OF VARIOUS GLUCOAMYLASES IN TERMS OF OPTIMUM pH, OPTIMUM TEMPERATURE AND MOLECULAR WEIGHT

Optimum pH	Optimum Temp. .degree.C..sup.(a)	Molecular Weight.sup.(a)	Glucoamylase
4.0*	75*	31,000*	(Talaromyces) Humicola lanuginosa.sup.(b)
6.5	65	--	Aspergillus luchensis.sup.(c)
4.0	65	--	Aspergillus niger
4.5*	70*	97,000.sup.(d)	Rhizopus sp.
5.0*	60*	70,000.sup.(e)	Endomyces sp..sup.(f)
5.0	60	64,000	Trichoderma viride.sup.(g)
5.0	60	75,000	Cephalosporium cherticola.sup.(h)
5.4	60	69,000	

.sup.(a) All values except those marked with an asterisk (*) were taken from the references. .sup.(b) P. M. Taylor et. al.: Carbohydrate Research 61,301 (1978). .sup.(c) T. Kanno et. al.: Public Notice of Japanese Patent Sho 53 (1978)7513. .sup.(d) J. H. Pazur, et. al.: J. Biol Chem. 237, 1002 (1962). .sup.(e) Hiromi et al.: Biochem Biophys. Acta 302,632 (1973). .sup.(f) Hattori et. al.: Agr. Biol. Chem 25,895 (1961). .sup.(g) Okada: J. Jap. Soc. Starch Sci. 21,282 (1974). .sup. (h) H. Urbanek: Appl. Microbiol 30, 163 (1975).

Claims Text - CLTX (1):

1. A process for producing a glucoamylase enzyme preparation which comprises culturing cells of a strain of Talaromyces .[.duponti.]. .ladd.leycettanus .laddend.in a nutrient medium and isolating the glucoamylase enzyme preparation from the culture medium.

US-PAT-NO: 4536477

DOCUMENT-IDENTIFIER: US 4536477 A

TITLE: Thermostable glucoamylase and method for its production

DATE-ISSUED: August 20, 1985

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Katkocin; Dennis M.	Danbury	CT	N/A	N/A
Word; Nancy S.	Woodridge	IL	N/A	N/A
Yang; Shiow-Shong	Downers Grove	IL	N/A	N/A

APPL-NO: 06/ 524070

DATE FILED: August 17, 1983

US-CL-CURRENT: 435/205, 435/842 , 435/96

ABSTRACT:

This invention relates to a glucoamylase enzyme exhibiting thermostability at pH values between 6 and 7 which is derived from a spore-forming, thermophilic, anaerobic bacterium and to a process for its production. The glucoamylase is especially useful for the preparation of glucose-containing syrups from starch.

9 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Detailed Description Text - DETX (29):

The thermostability of the purified glucoamylase was compared with that of two other known glucoamylases. The purified enzyme was diluted with sodium acetate buffer (100 mM, of the desired pH) to give a protein concentration of 12 .mu.g/ml. The enzyme solutions were incubated at 70.degree. C. At appropriate time intervals (usually 5, 10, 20, 30, 60 and 120 minutes), vials were removed and immediately cooled in an ice bath. Residual enzyme activity was assayed at pH 5 and 60.degree. C. using the standard assay procedure. The half-life of the enzyme was calculated by linear regression. Results given in Table II indicate that the enzyme of the present invention shows superior stability at 70.degree. C. and pH 5 or 6 over the glucoamylases produced by Talaromyces duponti and Aspergillus niger. It has a half-life of over 3 hours at pH 6 and 70.degree. C.

US-PAT-NO: 4318989

DOCUMENT-IDENTIFIER: US 4318989 A

TITLE: Starch-degrading enzymes from Cladosporium resinae

DATE-ISSUED: March 9, 1982

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Marshall; James J.	Miami	FL	N/A	N/A

APPL-NO: 06/ 159949

DATE FILED: June 16, 1980

PARENT-CASE:

This is a division of application Ser. No. 046,482 filed June 7, 1979, now U.S. Pat. No. 4,234,686 issued Nov. 18, 1980, which in turn is a continuation-in-part of application Ser. No. 892,747 filed Apr. 3, 1978, now U.S. Pat. No. 4,211,842 issued July 8, 1980.

US-CL-CURRENT: 435/205, 435/210

ABSTRACT:

A culture filtrate of Cladosporium resinae (Strain ATCC No. 20495) has been found to contain a mixture of starch-degrading enzymes capable of bringing about efficient conversion of starch and pullulan into glucose. Culture conditions resulting in optimal production of the pullulan degrading activity been established. The amylolytic enzyme preparation obtained by culturing the fungus under these optimal conditions has been fractionated by ion-exchange and molecular sieve chromatography and shown to contain at least four enzymes, a maltase, .alpha.-amylase and two glucoamylase-type enzymes including a novel exo-pullulanase. The maltase and glucoamylase enzymes have been purified to homogeneity and their substrate specificity investigated. Both the mixture and the exo-pullulanase can be used in the manufacture of dextrose from starch. Alone or in conjunction with appropriate other starch-degrading enzymes the mixture or the exo-pullulanase may be used in the production of specific starch conversion products, including fructose syrups. Both the mixture and the exo-pullanase are useful in increasing the fermentability of high DE starch conversion products and may be useful in the production of low calorie alcoholic beverages.

2 Claims, 11 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 11

---------- KWIC ---------

Detailed Description Text - DETX (24):
Table IV sets forth the purification procedure in detail for glucoamylase S and exo-pullulanase and indicates the increasing specific activity obtained

during purification.

Detailed Description Text - DETX (35):

An amount of the enzyme, either the mixture or the exo-pullulanase, sufficient to convert substantially all of the starch, is added to the solubilized starch. The exact amount of either material which is required depends upon the enzyme preparation used, although amounts in the range 1-30 International Units of activity per milliliter of reaction mixture are usually employed (1 International Unit of activity is the amount that releases 1 .mu.mole of glucose per minute at 37.degree. C. under optimal conditions of starch degradation). For the mixture, use of a final concentration of 5 International Units of activity per milliliter of reaction mixture will typically bring about 95-100% conversion in about 20 hours. Suitable temperatures are in the range 30.degree.-70.degree. C., preferably less than about 55.degree. C., because the enzyme is slightly less thermostable than the Aspergillus enzyme. The enzyme preparation can be modified by immobilization or other means in such a way as to increase thermostability, e.g. on DEAE-cellulose or inorganic carriers. After the conversion is complete the process is completed by standard procedures, e.g., enzyme inactivation, decolorization, crystallization, and removal of the crystallized enzyme.

US-PAT-NO: 4318927

DOCUMENT-IDENTIFIER: US 4318927 A

TITLE: Method of producing low calorie alcoholic beverage with starch-degrading enzymes derived from Cladosporium resinae

DATE-ISSUED: March 9, 1982

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Marshall; James J.	Miami	FL	N/A	N/A

APPL-NO: 06/ 159950

DATE FILED: June 16, 1980

PARENT-CASE:

This is a division of application Ser. No. 046,482 filed June 7, 1979, now U.S. Pat. No. 4,234,686 issued Nov. 18, 1980, which in turn is a continuation-in-part of application Ser. No. 892,747 filed Apr. 3, 1978, now U.S. Pat. No. 4,211,842 issued July 8, 1980.

US-CL-CURRENT: 426/11, 426/16

ABSTRACT:

A culture filtrate of Cladosporium resinae (Strain ATCC No. 20495) has been found to contain a mixture of starch-degrading enzymes capable of bringing about efficient conversion of starch and pullulan into glucose. Culture conditions resulting in optimal production of the pullulan degrading activity been established. The amylolytic enzyme preparation obtained by culturing the fungus under these optimal conditions has been fractionated by ion-exchange and molecular sieve chromatography and shown to contain at least four enzymes, a maltase, .alpha.-amylase and two glucoamylase-type enzymes including a novel exo-pullulanase. The maltase and glucoamylase enzymes have been purified to homogeneity and their substrate specificity investigated. Both the mixture and the exo-pullulanase can be used in the manufacture of dextrose from starch. Alone or in conjunction with appropriate other starch-degrading enzymes the mixture or the exo-pullulanase may be used in the production of specific starch conversion products, including fructose syrups. Both the mixture and the exo-pullanase are useful in increasing the fermentability of high DE starch conversion products and may be useful in the production of low calorie alcoholic beverages.

4 Claims, 11 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 11

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Detailed Description Text - DETX (24):

Table IV sets forth the purification procedure in detail for glucoamylase S and exo-pullulanase and indicates the increasing specific activity obtained during purification.

Detailed Description Text - DETX (35):

An amount of the enzyme, either the mixture or the exo-pullulanase, sufficient to convert substantially all of the starch, is added to the solubilized starch. The exact amount of either material which is required depends upon the enzyme preparation used, although amounts in the range 1-30 International Units of activity per milliliter of reaction mixture are usually employed (1International Unit of activity is the amount that releases 1 .mu.mole of glucose per minute at 37.degree. C. under optimal conditions of starch degradation). For the mixture, use of a final concentration of 5 International Units of activity per milliliter of reaction mixture will typically bring about 95-100% conversion in about 20 hours. Suitable temperatures are in the range 30.degree.-70.degree. C., preferably less than about 55.degree. C., because the enzyme is slightly less thermostable than the Aspergillus enzyme. The enzyme preparation can be modified by immobilization or other means in such a way as to increase thermostability, e.g. on DEAE-cellulose or inorganic carriers. After the conversion is complete the process is completed by standard procedures, e.g., enzyme inactivation, decolorization, crystallization, and removal of the crystallized enzyme.

US-PAT-NO: 4254225

DOCUMENT-IDENTIFIER: US 4254225 A
See image for Certificate of Correction

TITLE: Novel neutral glucoamylase and method for its production

DATE-ISSUED: March 3, 1981

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tamura; Masaki	Kamakura	N/A	N/A	JP
Shimizu; Mizuho	Hino	N/A	N/A	JP
Tago; Minoru	Tokyo	N/A	N/A	JP

APPL-NO: 06/ 055717

DATE FILED: July 9, 1979

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	53-87812	July 20, 1978

US-CL-CURRENT: 435/96, 435/205 , 435/911

ABSTRACT:

Process for the production of a glucoamylase having a pH optimum at about 6.0 to 6.5 by a strain of Stachybotrys and the glucoamylase produced thereby.

11 Claims, 4 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 4

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Brief Summary Text - BSTX (2):

At present, when producing dextrose industrially from starch, the principal glucoamylases employed for the saccharification process are those produced by microorganisms belonging to the genera Rhizopus and Aspergillus. The conditions under which these glucoamylases are employed are pH 5.0 and 55.degree. C. for the enzyme of the Rhizopus microorganism, and pH 4.5 and 60.degree. C. for the Aspergillus microorganism's enzyme. In addition, maximum dextrose content of the hydrozylate is about 96% (dry solids basis) when these glucoamylases react with enzyme liquefied starch at a 30% concentration. One reason that the dextrose yield does not reach 100% is that isomaltose accumulates due to a reverse reaction by these glucoamylases. However, there was recently published a report (U.S. Pat. No. 3,897,305) that the reverse reaction of glucoamylses is extremely small in the vicinity of neutrality and that the dextrose yield can thus be elevated to about 98% by carrying out the reaction at about a neutral pH with the joint use of pullulanase. The pullulanase acts to debranch the starch and increases the rate of glucoamylase action under these nearly neutral conditions. As far as neutral glucoamylases are concerned, only one has been reported to date, that

being the glucoamylase produced by the rice blast-causing fungus (Piricularia oryzae; Kazuo Matsuda, et al: Amylase Symposium, Vol. 9, 1974), but this glucoamylase possesses low thermostability and so cannot be employed under industrial conditions.

Brief Summary Text - BSTX (5):
It is another object of the invention to provide a glucoamylase that possesses enough thermostability so that it can be employed under industrial reaction conditions.

Brief Summary Text - BSTX (7):
A microbial strain has been discovered which produces a new glucoamylase having optimum activity at a pH of 6.0 to 6.5 and good thermostability. The new glucoamylase is capable of converting a 30% by weight solution of a 10 D.E. (dextrose equivalent) liquefied starch to a product containing at least about 96% dextrose when reacted with the starch hydrolyzate at pH 6.0 to 6.5 at 55.degree. C. This invention includes the method for the production of this glucoamylase wherein the microorganism of the genus Stachybotrys, which produces the glucoamylase, is cultured in a medium and the enzyme is recovered from the culture broth.

Drawing Description Text - DRTX (5):
FIG. 4 provides a comparison of the present enzyme and the conventional glucoamylases produced by the R. niveus, A. niger and P. oryzae microorganisms in terms of their relative thermostabilities.

Detailed Description Text - DETX (7):
The present enzyme is able to hydrolyze such carbohydrate compounds as starch, soluble starch, amylose, amylopectin and glycogen, and to produce dextrose from them. The yield of dextrose from each of these substrates is 100% when the substrate concentration is 1%. The mutarotation of the produced dextrose is positive. This enzyme is thus a glucoamylase. The reaction velocity of this enzyme was compared to the rates shown by the glucoamylases produced by microorganisms belonging to Rhizopus and Aspergillus in relation to various substrates. The results are presented in Table I. As can be seen from this table, the activity of the present enzyme is notably higher than the activities of the other two glucoamylases especially in relation to the hydrolysis of pullulan.

Detailed Description Text - DETX (15):
FIG. 3 presents inactivation curves of the relative enzymatic activity of the present enzyme when it was treated for 60 minutes at 60.degree. C. over a pH range of 3 to 8. As is clear from the figure, this enzyme is most stable at pH 6, and it is completely inactivated by this treatment for 30 minutes at pH 3 and for 1 hour at pH 4. In addition, FIG. 4 shows a comparison of the thermostability of the present enzyme and the glucoamylases from the Rhizopus, Aspergillus and Piricularia microorganisms. Namely, FIG. 4 presents the inactivation curves obtained for these enzymes when they were treated at 60.degree. C. while being held at their respective optimum pH's for stability. It can be seen that the thermostability of the present enzyme is inferior to that of the glycoamylase of Aspergillus origin, but is superior to the thermostability shown by the glucoamylases from the Rhizopus and the Piricularia microorganisms.

Detailed Description Text - DETX (24):
Regarding the optimum pH of enzymes, it can be seen from the data presented in FIG. 1 and Table II that the only glucoamylases which have their optimum pH's near the neutral zone are the present enzyme and the glucoamylase produced by the rice blast microorganism, Piricularia oryzae. However, as is clear from

FIG. 2 and Table II, the present enzyme and the rice blast glucoamylase have optimum reaction temperatures which are extremely different. In addition, the curves presented in FIG. 4 indicate that the thermostability of the present enzyme is vastly superior to that of the rice blast glucoamylase. Moreover, Table II shows that the molecular weight of the present enzyme is much smaller than the molecular weight of the other known glucoamylases

US-PAT-NO: 4247637

DOCUMENT-IDENTIFIER: US 4247637 A
See image for Certificate of Correction

TITLE: Highly thermostable glucoamylase and process for its production

DATE-ISSUED: January 27, 1981

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tamura; Masaki	Kamakura	N/A	N/A	JP
Shimizu; Mizuho	Hino	N/A	N/A	JP
Tago; Minoru	Tokyo	N/A	N/A	JP

APPL-NO: 06/ 055723

DATE FILED: July 9, 1979

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	53-106354	September 1, 1978

US-CL-CURRENT: 435/96, 435/205 , 435/911

ABSTRACT:

Process for the production of a thermostable glucoamylase by a strain of Talaromyces and the glucoamylase produced thereby.

8 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

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Abstract Text - ABTX (1):
Process for the production of a thermostable glucoamylase by a strain of Talaromyces and the glucoamylase produced thereby.

Brief Summary Text - BSTX (7):
A microbial strain has been discovered belonging to the genus Talaromyces which produces a glucoamylase having an optimum reaction temperature of 75.degree. C. and characterized as being capable of retaining at least about 90% of its initial glucoamylase activity when held at 70.degree. C. and pH 4.5 for 10 minutes. This invention includes the method for the production of this glucoamylase wherein a microorganism of the genus Talaromyces, which produces the glucoamylase, is cultured in a medium and the enzyme is recovered from the culture broth.

Detailed Description Text - DETX (54):
The strain, Talaromyces duponti G45-632, is one of the embodiments of the microorganism used in this invention and any microorganism belonging to genus

Talaromyces which is capable of producing the above-mentioned novel thermophilic glucoamylase can be employed as well as the strain G45-632 and its mutant strains.

Detailed Description Text - DETX (65):

A liquid culture medium consisting of 5% soluble starch, 2% corn steep liquor, 0.5% cottonseed meal, 0.5% yeast extract, 0.1% dipotassium phosphate, 0.05% magnesium sulfate and 0.01 calcium chloride was adjusted to pH 7.0 and 100 ml of this was placed in a 500 ml Erlenmeyer flask. This medium was sterilized at 121.degree. C. for 20 minutes, inoculated with Talaromyces duponti strain G45-632 and incubated at 40.degree. C. for 7 days on a shaker. After the culture was completed, the mycelia were removed from the culture fluid by filtration. The filtrate was found to contain 60 units of glucoamylase activity per milliliter.

Detailed Description Paragraph Table - DETL (1):

TABLE I _____ COMPARISON OF VARIOUS GLUCOAMYLASES IN TERMS OF OPTIMUM pH, OPTIMUM TEMPERATURE AND MOLECULAR WEIGHT Optimum Optimum Temp. Molecular Glucoamylase pH.sup.(a) .degree.C..sup.(a) Weight.sup.(a) _____ Present Enzyme 4.0* 75* 31,000* (Talaromyces) Humicola lanuginosa.sup.(b) 6.5 65 -- Aspergillus luchuensis.sup.(c) 4.0 65 -- Aspergillus niger 4.5* 70* 97,000.sup.(d) Rhizopus sp. 5.0* 60* 70,000.sup.(e) Endomyces sp..sup.(f) 5.0 60 64,000 Trichoderma viride.sup.(g) 5.0 60 75,000 Cephalosporium cherticola.sup.(h) 5.4 60 69,000 _____

.sup.(a) All values except those marked with an asterisk (*) were taken from the references. .sup.(b) P. M. Taylor et al.: Carbohydrate Research, 61, 301 (1978). .sup.(c) T. Kanno et al.: Public Notice of Japanese Patent Sho 53 (1978)7513. .sup.(d) J. H. Pazur, et al.: J. Biol. Chem. 237, 1002 (1962). .sup.(e) Hiromi et al.: Biochem. Biophys. Acta 302, 362 (1973). .sup.(f) Hattori et al.: Agr. Biol. Chem. 25, 895 (1961). .sup.(g) Okada: J. Jap. Soc. Starch Sci. 21, 282 (1974). .sup.(h) H. Urbanek: Appl. Microbiol. 30, 163 (1975).

Claims Text - CLTX (1):

1. A process for producing a glucoamylase enzyme preparation which comprises culturing cells of a strain of Talaromyces duponti in a nutrient medium and isolating the glucoamylase enzyme preparation from the culture medium.

Claims Text - CLTX (7):

7. The process of claim 6 wherein the glucoamylase is obtained from the strain of Talaromyces duponti, Fermentation Research Institute, Deposit No. 4566.

US-PAT-NO: 4234686

DOCUMENT-IDENTIFIER: US 4234686 A

TITLE: Starch-degrading enzymes derived from cladosporium resinae

DATE-ISSUED: November 18, 1980

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Marshall; James J.	Miami	FL	N/A	N/A

APPL-NO: 06/ 046482

DATE FILED: June 7, 1979

PARENT-CASE:

This is a continuation-in-part of Application Ser. No. 892,747 filed Apr. 3, 1978, the disclosure of which is hereby incorporated by reference into the present disclosure.

US-CL-CURRENT: 435/94, 435/96 , 435/98 , 435/99

ABSTRACT:

A culture filtrate of Cladosporium resinae (Strain ATCC No. 20495) has been found to contain a mixture of starch-degrading enzymes capable of bringing about efficient conversion of starch and pullulan into glucose. Culture conditions resulting in optimal production of the pullulan degrading activity been established. The amylolytic enzyme preparation obtained by culturing the fungus under these optimal conditions has been fractionated by ion-exchange and molecular sieve chromatography and shown to contain at least four enzymes, a maltase, .alpha.-amylase and two glucoamylase-type enzymes including a novel exo-pullulanase. The maltase and glucoamylase enzymes have been purified to homogeneity and their substrate specificity investigated. Both the mixture and the exo-pullulanase can be used in the manufacture of dextrose from starch. Alone or in conjunction with appropriate other starch-degrading enzymes the mixture or the exo-pullulanase may be used in the production of specific starch conversion products, including fructose syrups. Both the mixture and the exo-pullanase are useful in increasing the fermentability of high DE starch conversion products and may be useful in the production of low calorie alcoholic beverages.

8 Claims, 11 Drawing figures

Exemplary Claim Number: 1,7

Number of Drawing Sheets: 11

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Detailed Description Text - DETX (25):
Table IV sets forth the purification procedure in detail for glucoamylase S and exo-pullulanase and indicates the increasing specific activity obtained

during purification.

Detailed Description Text - DETX (36):

An amount of the enzyme, either the mixture or the exo-pullulanase, sufficient to convert substantially all of the starch, is added to the solubilized starch. The exact amount of either material which is required depends upon the enzyme preparation used, although amounts in the range 1-30 International Units of activity per milliliter of reaction mixture are usually employed (1 International Unit of activity is the amount that releases 1 .mu.mole of glucose per minute at 37.degree. C. under optimal conditions of starch degradation). For the mixture, use of a final concentration of 5 International Units of activity per milliliter of reaction mixture will typically bring about 95-100% conversion in about 20 hours. Suitable temperatures are in the range 30.degree.-70.degree. C., preferably less than about 55.degree. C., because the enzyme is slightly less thermostable than the Aspergillus enzyme. The enzyme preparation can be modified by immobilization or other means in such a way as to increase thermostability, e.g. on DEAE-cellulose or inorganic carriers. After the conversion is complete the process is completed by standard procedures, e.g., enzyme inactivation, decolorization, crystallization, and removal of the crystallized enzyme.